Baculovirus biopesticides - a safe alternative to chemical protection of plants

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ABSTRACT

Chemical pest control agents, though extensively used in all countries of the world, have been widely regarded as ecologically unacceptable. Therefore, there is the increased social pressure to replace them gradually with biopesticides which are safe to humans and non-target organisms. Viruses of a few families infect invertebrates but only those belonging to the family *Baculoviridae* have been used as biopesticides because they are safe to wildlife and their specificity is very narrow. Until recently, the application as bioinsectides was limited because of their slow killing action and technical problems for *in vitro* commercial production. However, successful protection of large area of soybean fields in Brazil revived the interest in baculoviruses as effective agents for biocontrol and the wider application for pest control is very likely to occur in future. To improve baculovirus killing properties, two approaches can be foreseen: i) in countries where use of genetically modified organisms is restricted, changes in biopesticide formulations and the improvements of the *in vitro* production are to be expected, ii) in countries with more relaxed attitude towards genetically modified organisms, the killing activity of baculoviruses will be improved by genetic modifications of the baculovirus genome.

Key words: *Baculovirus*, biopesticides, NPV, GV

BIOLOGICAL AND MOLECULAR PROPERTIES OF BACULOVIRUSES

Insects have many pathogens which include bacteria, fungi, nematodes and viruses. All of them effectively suppress pests when applied artificially as microbial pesticides (Vasantharaj, 2008; Saxena, 2008). This type of biological control can be potentially permanent. The natural enemies supplied from outside may establish themselves in the pest population and exert long-term protection.

Bacterial pesticides are probably the most widely used and cheaper than the other methods of pest bioregulation. Insects can be infected with many species of bacteria but those belonging to the genus *Bacillus* are most widely used as pesticides. One of the *Bacillus* species, *Bacillus thuringiensis*, has developed many molecular mechanisms to produce pesticidal toxins; most of toxins are coded for by several *cry* genes (Schnepf *et al.*, 1998). At the end of the twentieth century worldwide sales of bacterial pesticides amounted to about 2% of the total global insecticide market but their share in pesticide market steadily increases. Out of fifteen families of viruses known to infect insects, only those belonging to family *Baculoviridae* have been used as pesticides (Copping and Menn, 2000; Lacey *et al.*, 2002). Members of this family are regarded as safe to vertebrates. Their specificity is usually very narrow, often limited to single insect species. They have been used in many countries but their application as pesticides was limited in the past. Wider use of baculoviruses as commercial insecticides was restricted because of their slow killing action and difficulties in *in vitro* large scale production. This has changed when a very successful project was carried out in Brazil (Moscardi, 1999); over 2.0 million hectare of soybean had been already controlled annually by velvetbean caterpillar baculovirus. Following this successful project, many countries have increased the area of fields and forests protected by baculovirus pesticides.

The genome of baculoviruses is built of a double-stranded DNA ranging from 80 to about 200 kbp in length. In the past the classification of the family *Baculoviridae* was based on virus morphology. It was divided into two genera: the *Nucleopolyhedrovirus* (NPVs) and the *Granulovirus* (GVs). A new division was recently been proposed (Jehle *et al.*, 2006) on the basis of comparison of genomic sequences which indicated that virus phylogeny followed more closely the classification of the hosts than the virion morphological traits. In the classification the family *Baculoviridae* contains four genera: *Alphabaculovirus* (lepidopteran-specific NPVs), *Betabaculovirus* (lepidopteran-specific GVs), *Gammaba culovirus* (hymenopteran-specific NPVs), and *Deltabacul ovirus* (dipteran-specific NPVs). Baculo

viruses infect only arthropods and they do not replicate in vertebrates, plants and microorganisms. However, though they do not replicate, they may, under special conditions, enter animal cells. After the entrance to mammalian cells they are rapidly inactivated. This property made them a valuable tool for studies of expression of foreign genes under vertebrate promoters introduced into baculovirus genome (Kost *et al.*, 2005).

Individual baculoviruses usually have a narrow host range limited to a few closely related species. The viral progeny is present as occlusion bodies in infected cells which allow virus to survive in the environment and to transmit the disease from one insect to another. Virions consist of one or more nucleocapsids embedded in an membrane envelope containing virus-encoded protein. On their surface, mature virions have an additional covering of proteins and carbohydrate, known as polyhedron envelope or calyx. The most widely studied baculovirus is the *Autographa californica* nucleopolyhedrovirus (AcMNPV). The circular DNA genome of AcMNPV is surrounded by a small basic protein which neutralizes the negative charge of the DNA. This structure is protected by proteins forming a nucleocapsid. The genomic circular DNA is infectious in the naked form. Two genetically identical but morphologically distinct viral forms are produced at different stages of infection. Budded virus particles (BV) serve for the transmission of the virus to other tissues of the caterpillar body. Occlusion bodies (OB) are responsible for the survival of the virus in the environment and the spread of the virus from insect to insect. The occlusion bodies (polyhedra) contain many occlusion-derived virions (ODV) surrounded by a matrix composed mainly of polyhedrin, a major structural protein (Braunagel *et al.*, 2003). Polyhedra are stable and the virions can survive in the environment for years if they are in the favorable conditions. They are big enough to be seen in a light microscope. Under magnification of around 1000x, polyhedra resemble clear, irregular crystals (Bonning, 2005). Caterpillars ingest polyhedra together with their food. The polyhedrin matrix is solubilized in the alkaline pH of the midgut and the released virions enter midgut cells after fusion with microvilli (Fig.1). The virions are uncoated and nucleocapsids enter the nucleus where viral genes are expressed in strictly controlled manner. Late AcMNPV genes are transcribed between 6 and 24 hours post infection, while very late genes began to be transcribed around 18 hours post infection. and the transcription continues until 72 hours post infection (Lu and Miller, 1997). In the late phase nucleocapsid structural proteins are synthesized, including glycoprotein GP64 which plays an important role in the horizontal infection by budded virus. In the very late phase the production of infectious BVs is greatly reduced. Nucleocapsids become enveloped usually in groups of a few particles. This process appears to be an essential primary step in the process of occlusion of nucleocapsids by the very late protein – polyhedrin. The process of occlusion lasts until the nucleus becomes filled with occlusion bodies as a consequence, around 10^{10} polyhedra are produced per larvae which may account for over 30% of the dry weight of a caterpillar (Miller *et al.*, 1983). During the process of occlusion there is an accumulation of fibrillar structures in cells. They are composed mostly of a very late protein p10. These structures are probably involved in the disintegration of the host cells (Van Oers *et al.*, 1994). In the final stages of infection, two viral enzymes, chitinase and cathepsin, are crucial for the host cuticle breakdown (Hawtin *et al.*, 1997). Finally the larvae liquifies. Polyhedra released from the dead larvae can infect other caterpilars by horizontal transmission which primarily occurs through larvae ingesting occlusion bodies present on leaves. The occlusion bodies can be further distributed by excrements of infected larvae and predators (Vasconselos., 1996). Vertical transmission may also play a role in spreading the virus. It usually takes place through contamination of eggs or virus entering inside the egg (Fuxa *et al.*, 2002).

SUCCESSES AND FAILURES OF BACULOVIRUS PESTICIDES IN THE PAST

First well-documented introduction of baculovirus into the environment which resulted in effective suppression of a pest occurred accidentally before the World War II. Along with a parasitoid imported to Canada to suppress spruce sawfly *Diprion hercyniae*, an NPV specific for spruce sawfly was introduced and since then no control measures have been required against this hymenopteran species. The introduction of alien baculovirus, which becomes a permanent part of an ecosystem, is rather an exception. Usually two other strategies of pest management are employed (Fuxa, 2004):

- infested areas are sprayed with highly concentrated baculovirus to suppress the pest as quickly as possible,
- infested areas are sprayed with lower concentration of baculovirus and this results in establishment of the virus for more than one generation.

In the past, the application of baculoviruses for the protection of agricultural annual crops, fruit orchards and forests has not matched their potential. The number of registered pesticides based on baculovirus, though slowly, increases steadily. At present, it exceeds fifty formulations, some of them being the same baculovirus preparations distributed under different trade names in different countries.

NPVs and GVs are used as pesticides but the group based on nucleopolyhedrosis viruses is much larger.

The first viral insecticide Elcar™ was introduced by Sandoz Inc. in 1975 (Ignoffo and Couch, 1981). Elcar™ was a preparation of *Heliothis zea* NPV which is relatively broad-range baculovirus and infects many species belonging to genera *Helicoverpa* and *Heliothis*. HzSNPV provided control of cotton bollworm, but also of pests belonging to these genera attacking soybean, sorghum, maize, tomato and beans. In 1982 Sandoz decided to discontinue the production. The resistance to many chemical insecticides including pyrethroids revived the interest in HzSNPV and the same virus was registered under the name GemStar™. HzSNPV is a product of choice for biocontrol of *Helicoverpa armigera* (Mettenmeyer, 2002). Countries with large areas of such crops like cotton, pigeonpea, tomato, pepper and maize, e.g. India and China, introduced special programs for the reduction of this pest by biological means. In Central India, *H.armigera* in the past was usually removed by shaking pigeonpea plants until caterpillars fell from the plants onto cotton sheets. This technique is now used to obtain caterpillars which are fed on virus-infected seeds. Baculovirus preparations obtained in this way are used by farmers to prepare a bioinsecticide spray applied on pigeonpea fields. Another baculovirus, HaSNPV is almost identical to HzSNPV. It was registered in China as a pesticide in 1993 (Zhang *et al.*, 1995). It has been used for large scale biopesticide production and has been extensively used on cotton fields (over 100 000 ha of cotton in the last decade). Broad spectrum of biopesticide based on HaNPV is also used in India (Srinivasa *et al*., 2008).

The forests of temperate regions are very often attacked and defoliated by moths of Lymantridae and Noctuidae families (most common pest species are: *Lymantria dispar, Lymantria monacha*, *Orgiya pseudotsugata* and *Panolis flammea*) and some Hymenoptera species (mainly *Neodiprion sertifer and Diprion pini*). *Limantria dispar* MNPV formulations marketed under trade names: Gypchek, Disparivirus, Virin-ENSH, and *O.pseudotsugata* MNPV under trade names: TM BioControl-1 and Virtuss (Reardon *et al.*, 1996) are sometimes used for forest protection. The area protected with these biopesticides at present is still marginal but gradually increases because their use is favoured by ecological concern on the part of forest managers.

Caterpillars of moths belonging to *Spodoptera* genus are of primary concern for agricultural industry in many countries of the world. Two commercial preparations based on *Spodoptera* NPV are available in the USA and Europe. These are SPOD-X™ containing *Spodoptera exigua* NPV to control insects on vegetable crops and Spodopterin™ containing *Spodoptera littolaris* NPV which is used to protect cotton, corn and tomatoes. About 20 000 hectares of maize annually are controlled with *Spodoptera frugiperda* NPV in Brazil (Moscardi, 1999). Use of *Spodoptera litura* NPV has been tested on cabbage crops in India (Kumari *et al.*, 2009). Many other species belonging to the Noctuidae family are economically important pests of sugarcane, legume, rice and others. *Autographa californica* and *Anagrapha falcifera* NPVs were registered in the USA and were field-tested at a limited scale. These two NPVs have relatively broad host spectrum and potentially can be used on a variety of crops infested with pests belonging to a number of genera, including *Spodoptera* and *Helicoverpa*.

Granulovirus CpGV is the active component of a number of biopesticides used for protection of orchards (Kutinkova *et al.*, 2008). Some of the trade marks of GpGV-based products are following: Granusal™ in Germany, Carpovirusine™ in France, Madex™ and Granupom™ in Switzerland, Virin-CyAP in Russia.. Annually up to 100 000 hectares of orchards has been protected with CpGV. Another granul ovirus, *Erinnyis ello* (cassava hornworm) granulovirus, was found to be very efficient for protection of cassava plantations (Bellotti, 1999). This GV has been used for spraying cassava crops in some South American countries. In Brazil a successful program to cassava pest control was carried out in the eighties based on recovering the virus that were multiplied in the field larval population. However, due to *Erinnyis ello* cyclical behaviour and the difficulty in the insect mass production in laboratory conditions, the program was discontinued.

The well-known success of employing baculovirus as a biopesticide is the case of *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) used to control the velvetbeen caterpillar in soybean (Moscardi, 1999). This program was implemented in Brazil in the early eighties, and came up to over 2,000,000 ha of soybean treated annually with the virus. Recently this number dropped down, mainly due to new emerging pests in the soybean complex. Although the use of this virus in Brazil is the most impressive example of bioregulation with viral pesticide worldwide, the virus is still obtained by *in vivo* production mainly by infection of larvae in soybean farms. The demand for virus production has increased tremendously for protection of four million hectares of soybean annually. This high demand for AgMNPV calls for the studies aiming at the sustained inexpensive *in vitro* production of the virus because large scale *in vivo* production of baculoviruses encounters many difficulties. The use of AgMNPV in Brazil brought about many economical, ecological and social benefits. At the soybean grower level, the financial savings from the use of the virus may reach up to ca. U$ 7/ha/season, including product cost and application cost. The current annual savings at the grower level, in the total area sprayed with the virus is over US$ 11,000.000. Since the

beginning of the program more than 17 million liters of chemical insecticides were not sprayed in the environment. The protection of soybean fields in Brazil has proven that baculoviral control agents can be effectively produced on a large scale and they may be an alternative to broad-spectrum chemical insecticides. On the basis of this spectacular success of a baculovirus pesticide, it is needless to say that the advantages of biopesticides over chemical pesticides are numerous. Safety for humans and non-target organisms, preservation of biodiversity in the environment, reduction of toxic residues in agricultural end-products are just the examples of potential benefits. However, the cost of biopesticide production has been usually higher than the cost of conventional pesticides. So, paradoxically, countries where the cost of human labour is low are more open towards the use of baculoviral pesticides than rich Western countries which claim that environmental protection is one of their priorities in the development.

Genomic variability has been described for many wild type virus including *Autographa californica* MNPV, *Spodoptera frugiperda* MNPV, *Spodoptera litura* MNPV, *Panolis flammea* MNPV and *Mamestra configurata* NPV. Genotypic variants can be recognized by the presence of submolar fragments in the electrophoretic patterns of restriction endonuclease digestion products of a viral genome. Genotypic variation in baculovirus genomes can include point mutations, both small and large deletions and insertions (Krell, 1996). Though mutations can occur in any place of the genome, the presence of some hot spots was observed for certain genomic alterations such as insertions due to transposable elements or deletions in the hypervariable DA26 gene region (Kamita *et al.*, 2003). AgMNPV genomic variability has been also carefully studied because the selection pressure due to the application of AgMNPV in the field during subsequent years could lead to alterations in virus stability. The method of choice was the technique of restriction endonuclease analysis. Viral DNA were initially purified from diseased larvae collected during several crop seasons and compared to AgMNPV-79, a wild-type virus that was used originally and subsequently in this program (Souza *et al.*, 2001). These results indicated that the virus maintains considerable stability, even with the existence of some genetic changes shown in the DNA restriction profiles. It has been also observed that the virus retains its pathogenecity throughout the years of its application.

PROGNOSES FOR THE DEVELOPMENT OF BACULOVIRUS PESTICIDES IN FUTURE

Many years of application of AgMNPV in Brazil has proved that the baculovirus protection at large scale is possible and can be done at relatively low cost. It is very likely that the growing awareness of the benefits of the environment-friendly pesticides will result in the reevaluation of the prospects for biological protection with baculovirus preparations. Future development of baculovirus pesticides will probably depend on the attitude of man-in-the-street towards genetically modified organisms. In countries where use of genetically modified organisms is restricted, only naturally occurring baculoviruses will be used for protection of crops. In this case the improvements will be at the level of diagnostics of infection, development of the *in vitro* cultures and changes in the formulations of the biopesticide. In countries which favour the introduction of genetically modified organisms, the improvements will be achieved by introduction of exogenous genes into baculovirus genome. In this way the killing activity of baculovirus preparations can be greatly enhanced.

Reliable assays for the progress of infection with baculovirus are necessary because the major problem in using biopesticide for crop protection is their slow action and lack of morphological changes in larvae in first stages of baculovirus propagation. Lack of such assays may incline agricultural services to use subsequent chemical means of protection which, from the ecological point of view, may be redundant. Fast and sensitive methods in diagnostics which are crucial in pest management with baculovirus can be roughly divided into immunological methods based on protein composition and content, and genome detection methods usually based on PCR techniques. The DNA detection methods will probably play a predominant role in future. They are relatively simple analytical methods giving precise information about occurrence and spread of the virus. Using specific primers, not only target larvae, but also vectors for baculovirus transfer – predating invertebrates and birds can be quickly analysed. PCR-based methods are so common nowadays in almost all analytical laboratories associated with pest management. For quantitative analyses, the real-time PCR required – light cyclers are relatively expensive now, but their prices decrease very quickly and it is very likely that they will soon become routine equipment in pest management laboratories. One of the powerful methods for characterization of baculoviruses is single-strand conformational polymorphism. This technique allows for determining not only viral species but also viral strains usually without additional DNA sequencing. Classical single-strand conformational polymorphism analysis is based on the observation that single-stranded DNA fragments attain a number of conformational forms which may be separated by electrophoresis in a native polyacry lamide gel. For a fragment of a specific nucleotide sequence usually more than one conformational isoform is thermodynamically

stable and we observe a characteristic pattern of electrophoretic bands for a particular DNA sequence. Even minute sequence changes (e.g. a point mutation) may have significant effect on electrophoretic pattern of single-stranded DNA fragment. This method proved to be a very valuable tool in genotyping of many diseases in humans and animals and should find wider use in bioprotection (Kaczanowski *et al.,* 2001, Szewczyk *et al.,* 2008).

The *in vitro* production is still a strong requirement on a commercial perspective of baculoviruses use as insecticides. However the accumulation of genotypic variations by serial passage in cell culture prevents its large scale production. One of the most important effects of the viral passage is the change from the parental, many polyhedra per cell (MP) phenotype, to the few polyhedra per cell (FP) phenotype. The major problem of the passage effect is the reduced occlusion and loss of virulence of the occluded virus (Krell, 1996). Frequent mutations have been identified within a specific region in the Few Polyhedra mutants (FP) that contains the 25k *fp* locus (Harrison *et al.,* 1995; Bischoff and Slavicek,1997; Lua *et al.,* 2002). This gene encodes a 25KDa protein that is essential for virion occlusion and polyhedron formation. Another type of mutants generated during serial passage of baculovirus is the formation of Defective Interfering Particles (DIs). These mutants have lost the ability to be replicated in the host cell without the aid of a helper virus and large sizes of their genome are usually deleted (Pijlman *et al.,* 2005). These particles replicate faster because they are smaller, and inhibit the replication of a standard virus. The studies of morphological changes of AgMNPV due to the serial passages in cell culture as well as the analysis of susceptibility of different lepidopteran cell lines to AgMNPV are currently being carried out in Brazilian institutes in charge of AgMNPV programme (Castro *et al.,* 1997; Castro *et al.,* 1999; Rodas *et al.,* 2005; Castro *et al.,* 2006; Rezende *et al.,* 2009). The use of other baculoviruses requires similar studies if they have to be produced at large scale in *in vitro* conditions. In the years to come, the methods for production of baculovirus biopesticides in cell culture have to be improved to simplify commercial production of baculoviruses and to reduce the cost of large-scale production.

The stability of baculoviruses is influenced by temperature, pH, humidity, presence of additives but ultraviolet light is probably the most detrimental to viral survival. Under field conditions little activity is left when the virus is not shaded by plant canopy, therefore much effort has been devoted to the development of UV protectants (Shapiro and Dougherty, 1994; Zou and Young, 1994). The best results were obtained for stilbene fluorescent brighteners which are marketed under many trade names (e.g. Phorwite AR, Blankophor and others). Future developments in the formulations of brighteners may lead to the reduction of cost of baculovirus production. Inactivation of baculoviruses may be also caused by plant metabolites e.g. by peroxidases which generate free radicals (Hoover *et al.,* 1998). The inactivation can be reduced by addition of free radical scavengers such as mannitol or enzyme superoxide dismutase to baculovirus preparations (Zhou *et al.,* 2004).

The activity of baculoviruses against their natural hosts may be enhanced by interference with insect physiology or introduction of insect-specific toxins (Bonning and Hammock, 1996; Inceoglu *et al.,* 2001; Muraleedharan *et al.,* 2008). The changes to host physiology were done by introducing genes coding for some insect hormones or hormone-modifying enzymes into baculovirus genome, or by deletion of the baculovirus - encoded ecdysteroid glucosyltransferase (*egt*) gene. The former approach was employed by cloning juvenile hormone esterase gene into baculovirus genome which overexpressed decreases the concentration of the juvenile hormone and this is a signal for a caterpillar to stop feeding and pupate. This line of research is being pursued in some laboratories (Hammock *et al.,* 1990; Inceoglu *et al.,* 2001). The deletion of the baculo virus-encoded *egt* gene was used first by O'Reilly and Miller, 1991. The product of the *egt* gene interacts with larval moulting and indirectly increases the time of feeding of infected caterpillars. The *egt*-deletion from baculovirus genome resulted in 30% faster killing of caterpillars. Another advantage of this genomic modification is the fact that the *egt* gene is not essential for viral replication and can be replaced with an exogenous gene; the product of which may enhance the insecticidal activity of the recombinant virus (Sun *et al.,* 2004)

Baculovirus genome modifications by introduction of exogenous toxin genes were extensively studied in many laboratories. Most of the research was devoted to the studies of arthropod toxin genes isolated from scorpions or spiders (Bonning and Hammock,1996; Inceoglu *et al.,* 2001). The most potent insect-specific toxin gene used for construction of baculovirus recombinants was the gene coding for a toxin from scorpion *Androctonus australis*. The feeding damage caused by larvae infected with this modified baculovirus was reduced by about 60% in comparison to a wild type baculovirus (Inceoglu *et al.,* 2001). Toxin genes isolated from other scorpions, e.g. *Leiurus quinquestriatus hebraeus* (Froy *et al.,* 2000), straw itch mite *Pyemotes tritici* (Burden *et al.,* 2000), ants (Szolajska *et al.,* 2004) or spiders (Hughes *et al.,* 1997) have been intensively studied as potential enhancers of baculovirus activity. Arthropod toxins usually attack insect sodium

channels producing final effect similar to the chemical pesticides of the pyrethroid group. However, the specific target in sodium channels is different, so there is a potential possibility to produce synergistic effect of biopesticide/ chemical pesticide application is necessary (McCutchen *et al.*, 1997).

Baculovirus recombinants that produced occlusion bodies incorporating *Bacillus thuringiensis* toxin were constructed by making a fusion protein consisting of polyhedrin and Bt toxin (Chang *et al.*, 2003). The pathogenicity of the recombinant was remarkably increased compared to wild-type virus. These studies proved that it is possible to construct a biopesticide which combines the advantages of the virus and the bacterial toxin. The genomic modifications of baculovirus genome presented above proved to be very successful but the reluctant attitude of many countries towards genetically engineered organisms was a reason for a slow pace of the transfer of a potential biopesticide from a laboratory to industry. This negative approach to recombinant baculoviruses was not supported by scientific evidence. On the contrary, the scientific data indicate that baculoviruses pose no hazard to other animals than their hosts and this was documented by a number of studies from different laboratories. Recombinant baculoviruses were not pathogenic to bees and all vertebrate species (Sun *et al.*, 2004). The theoretical possibility of the cloned gene to jump from recombinant baculovirus to other organisms has not been proved (Inceoglu *et al.*, 2001). However, in spite of this sound evidence, preliminary field trials of genetically modified baculoviruses raised massive public protests which put on hold further trials for a long time. The responsibility for the slow progress in application of genetically modified baculoviruses as pesticides is in part due to scientists themselves who did not take into account the perception of their experiments by general public and modified baculovirus genomes by insertions of genes coding toxins from scorpions or spiders. These exciting scientific experiments were strongly criticized by media with high impact on an average, non-specialist, citizen in rich Western democratic countries. Taking into account these conflicts between scientists and men-in-the-street, we are of the opinion that the genetic modification of baculoviruses is probably a right path to go but the choice of toxins used for this purpose should be re-examined and baculoviruses should be modified with genes coding for natural insect toxins, for example with genes coding for toxic polypeptides of parasitoid wasps which occur in the region infested by particular pests. The more rational approach is also needed in the social perception of dangers associated with genetically modified baculoviruses and here, we hope, scientists will take an active part in convincing the public on risks and benefits of genetically modified biological pesticides.

CONCLUSIONS

Baculoviral pesticides again come into light and it is very likely that in future their role will be more significant in agriculture and forestry. The development of the *in vitro* production is a very important requirement that could lead to an improvement of baculoviruses commercial use. The breakthrough in the use of baculovirus pesticides was the protection of soybean fields in Brazil which has proven that baculoviral control agents can be effectively produced on a large scale and they may be an alternative to broad-spectrum chemical insecticides. Hopefully, more rational approach will be gradually adopted towards microbial pesticides in the near future and short-term profits from chemical pesticides will not determine the fate of biopesticides.

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