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Bacculoviruses: A Novel Approach in Integrated Pest Management

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ABSTRACT: Overdependence and indiscriminate use of synthetic chemical insecticides has poisoned almost every component of the environment, besides leading to insecticide resistance among insects, secondary pest outbreaks, residue problems in consumable commodities and human health hazards. In recent years, the utmost emphasis is being placed on use of bio pesticides and other non-chemical methods to manage insect- pests. Much of the current research and attention is being emphasized on the use of Microbial pesticides such as viruses, fungi, bacteria etc. for integrated pest management. Viruses of few families are known to infect vertebrates but only those belonging to family Baculoviridae have been used as biocontrol agents or commercialized as biopesticides. Baculoviruses are self-perpetuating, host specific, safer to natural enemies and known for causing high epizootic levels and are known to regulate many insect populations in nature. Their host-specificity is very high, usually restricted to a single or a few closely related insect species. Baculoviruses based pesticides are compatible with integrated pest management strategies and the expansion of their application will significantly reduce the risks associated with the use of synthetic chemical insecticides. Baculoviruses are being considered as one of the viable options to chemical pesticides as they are target specific, least toxic to natural enemies besides environment friendly. This review attempts to give an overall picture of baculoviruses as eco-friendly potential control agents, in view of the recognition of hazards posed by the more commonly used chemical insecticides in recent years. Still now, commercialization of baculoviruses is a major challenge, therefore researchers and stakeholders give attention to optimize massive production of baculoviruses for integrated insect pest management.

Keywords: Baculoviruses, Biopesticides, Nuclear Polyhedrosis viruses, Granulosis viruses, Virions, Lepidoptera.

INTRODUCTION

Insect viruses are submicroscopic, obligate, intracellular pathogenic entities. The viruses belonging to fifteen families are pathogenic to insects, but those belonging to family Baculoviridae are covalently closed, double stranded DNA genomes that range in size from 82 to 180 kilobases (Herniou *et al.*, 2004, Lauzon *et al.*, 2004) and have almost exclusively been used as pesticides (Copping, 1998). The baculoviruses have been reported exclusively from arthropods; and family Baculoviridae contains diverse members and is divided into two genera Nucleopolyhedrosis virus (NPV) and Granulovirus (GV) based on their occlusion bodies (OB). The first accounts of baculoviruses pathology was associated with the silkworm, *Bombyx mori*, probably because its economic significance in silk production. The baculoviruses was exploited as pest-control agents around 1527, when the viral nature of 'Jaundice disease' of the silkworm, *Bombyx mori* was established by 1947 (Misra, 1998). This led to the opinion that these viruses were widespread in nature among economically important insect pests, thus could be successfully exploited against their management in varied agro-climatic zones. Baculoviruses are one of the largest and most diverse groups of viruses pathogenic only for insects mostly of the order Lepidoptera, Hymenoptera, Diptera, Neuroptera, Coleoptera, Trichoptera, Crustacea and mites (Herniou *et al.*, 2004). Baculoviruses, especially NPVs, have been the subject of extensive safety testing in accordance with many of the specifications that have been applied to safety precautions of chemical pesticides. Baculoviruses represent a viable alternative to the use of synthetic chemical insecticides as they often exhibit high virulence, have relatively narrow or specific host ranges, and virtually no vertebrate or environmental toxicity (Hunter-Fujita *et al.*, 1998). They are also widely used as expression vectors in biotechnology.

Morphological characterization of baculoviruses. Baculovirus-a virus encoded RNA Polymerase; in nature occur as virions occluded within proteinaceous crystal known as polyhedron. Baculoviruses are rod shaped and have double stranded DNA. The rod like shape of the virions led to the name "baculovirus" for this family of viruses from the Latin word "baculum", which refers to a cane, walking stick, or staff. The rod-shaped virions, 40-70 nm x 250-400 nm, comprising a lipoprotein envelope around a protein capsid containing DNA-protein core occluded in a proteinaceous occlusion body that can persist outside the host for a considerable period of time (Clark, 1956) must be ingested or vertically transmitted to cause infection (Cory and Myers, 2003). The capsid and core constitute the nucleo-capsid. The nucleocapsid may be found either enveloped singly (SNPV) or by lipoprotein membrane envelope/ multiple nucleocapsid (MNPV) are embedded within each envelop. In between 20 to 200 virions are embedded in the polyhedron matrix depending upon the species.

Genetic engineered (Recombinant baculoviruses) are constructed in two basic steps. In the first instance, heterologous gene is introduced into a baculovirus transfer vector. It consists of a bacterial replicon of amulticopy plasmid, a selection marker gene, promoter and terminator regions along with the flanking baculovirus sequences from non-essential locus, and either a multiple cloning site or a single unique restriction site downstream from a viral promoter. The origin of the promoters and flanking DNA is normally from one of the late genes i.e, polyhedronor p10 gene. The p10 gene coding for a protein is usually produced in large quantities later in the infection cycle. It is the main component of the fibrillar structures which accumulate in the nucleus and in the cytoplasm of infected cells.

Mode of transmission of baculoviruses. Baculoviruses are known for high epizootic levels and is naturally occurring, self-perpetuating, and safe to natural enemies due to host specificity and environment friendly. Baculoviruses specifically infect insect larvae and adult insects are not susceptible to them. The insect larvae while feeding on the plant foliage, accidently feed upon the polyhedral which then gets solubilized in the insect midgut, thereby releasing the virions. These virions replicate within the nuclei of epithelial cells lining the midgut to produce more virions which are released in a budded form or get occluded within the polyhedral late in the infection process. Tissue liquefaction and then rupture of these cells upon death of the infected larvae liberates masses of these polyhedral in the soil environment. From here, they are again ready to be ingested and infect their hosts. These virions containing polyhedral are highly stable persisting in the soil environment for many years. The germicidal action of UV radiation of sunlight and substrate environment has greater influence in reducing the potency of viruses to kill the pest. These viruses are specific and often highly virulent to their hosts. They are restricted in their pathogenicity to the class Insecta and are often genus or species specific.

Important families of Baculoviruses. Based on nature of genetic material (RNA or DNA) and its form (Single or Double stranded), the size and shape of virus particle or the presence of membrane/proteinaceous inclusion body around the virus particles, the following families of baculoviruses have been enlisted:

Different families of Baculoviruses along with their genome.

Sr. No.	Family	Nucleic acid	Sr. No.	Family	Nucleic acid
1.	Parvoviridae	SsDNA	10.	Reoviridae	DsDNA
2.	Iridoviridae	DsDNA	11.	Birnaviridae	DsDNA
3.	Baculoviridae	DsDNA	12.	Rhabdoviridae	SsDNA
4.	Poxviridae	DsDNA	13.	Togaviridae	SsDNA
5.	Polydnaviridae	DsDNA	14.	Flaviviridae	SsDNA
6.	Ascoviridae	DsDNA	15.	Bunyaviridae	SsDNA
7.	Nodaviridae	SsDNA		Ss- Single stranded Ds- Double stranded	
8.	Picomaviridae	SsDNA			
9.	Tetraviridae	SsDNA			

Classification of Baculoviridae. In early classification, two classless ODV (Occlusion derived virion) and BV (Budded virion) were recognized based on two types of virions which are structurally and biochemically different from each other from the infected cells (Henderson *et al*., 1974; Summers and Anderson 1972). The earlier classification placed Baculoviruses within a single family, Baculoviridae; this classification scheme placed all NPVs into two genus- Nucleopolyhedrovirus and Granuloviruses. Jehle *et al.* (2006) proposed a new classification and nomenclature for the genera within the baculovirus family which included four genera: Alphabaculovirus (Lepidopteran-specific NPV), Betabaculovirus (Lepidopteran-specific Granuloviruses), Gammabaculovirus (Hymenopteran-specific NPV) and Deltabaculovirus (Dipteran-specific NPV). On the basis of morphological, biological, and phylogenetic features, members of family Baculoviridae are now classified into the following four genera:

Alphabaculovirus: The genus had Nucleopolyhedroviruses that infect insects from order Lepidoptera. The occlusion derived virions (ODV) produced by members of this genus may contain one or many nucleocapsids per enveloped virion, which is not found among members of the other genera. Most of the described NPVs are found in this genus. The Alpha-baculovirus type species is *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV).

Betabaculovirus: This genus consists of the Granulosisviruses, all of which have been isolated from Lepidoptera. The type species for this genus is *Cydiapomonella* granulovirus (CpGV).

Gammabaculovirus*:* This genus consists of NPVs from *Neodiprion* spp. of sawflies. The genomes of gammabaculoviruses that have been sequenced to date tend to be significantly smaller than those of other baculoviruses and do not contain any genes encoding a BV envelope fusion protein, something found in baculoviruses from the other genera. These viruses form infections that are restricted to the midgut of their hosts. The type species for this genus is *Neodiprion lecontei* nucleopolyhedrovirus (NeleNPV).

Deltabaculovirus: This genus includes the NPV isolated from the mosquito, *Culex nigripalpus*. The viral occlusion matrix protein of this virus is significantly larger than those of the viruses from the other three genera, and appears to share no significant amino acid sequence identity with other polyhedrins and granulins. The type species for Deltabaculovirus is *Culex nigripalpus* nucleopolyhedrovirus (CuniNPV).

Specificity of baculoviruses as bio pesticides. The specificity is most important character of baculoviruses as bio pesticides, as they are known to be completely safe to man, animals and important beneficial insects such as bees, predatory insects and parasitoids; and is compatible with other biological control options; moreover its use does not produce any of the secondary pest problems encountered when broad spectrum chemicals are used. Its specificity makes it safe to humans and can be sprayed onto crops right up to time of harvesting with no residue problems. Another important reason for interest in NPV as potential insect control agents is that, unusually for viruses they are relatively easy to see and count using a Light microscope. NPV are seen in dead/ dying larvae as bright irregular crystals called occlusion bodies (OB) or polyhedral inclusion bodies (PIB). Within these PIBs are found embedded up to 200 virus particles or virions. These virions are actual virus infective particles and are composed of rod-shaped DNA/protein structure called Nucleocapsid.

With the aim for effective insect pest management, emphasis has only been concentrated on those virus families that do not infect mammals. Mostly, baculoviruses infect arthropods and do not replicate in vertebrates, plants and other microorganisms. Though they do not replicate, but under special circumstances, they may enter animal cells. This unusual trait had made them a valuable tool in the recent past for studies of transient expression of foreign genes under vertebrate promoters introduced into baculovirus genome (Boyce and Bucher 1996; Kost *et al.*, 2005). The baculoviruses has been most studied as these are both highly infectious by ingestion and show good horizontal transmission (from adult to egg). They also have the advantage of infecting many important species of Lepidopteron and other insect pests.

Bio pesticide development is concerned almost exclusively with members of one family, the Baculoviridae (the Baculoviruses); which has two groups of viruses namely, Nucleopolyhedrosis virus (NPV) and Granulosis viruses (GV).

1. Nucleopolyhedrovirus (NPV) – Mostly contain several virions.

2. Granulovirus (GV) – normally contain a single virion. Both Madex Top and Capex contain granuloviruses.

1. Nucleopolyhedrovirus (NPV).

This baculovirus class classification is often combined with the name of the insect species the virus was originally isolated from to give the strain identification. For example, CpGV (*Cydiapomonella* Granulovirus) in Madex Top and AoGV (*Adoxophyes orana* Granulovirus) in Capex.

Identification: The NPV are seen in dead/ dying larvae as bright irregular crystals called occlusion bodies (OB) or Polyhedral Inclusion bodies (PIB). Within these PIBs are found embedded up to 200 virus particles or virions. These virions are actual virus infective particles and are composed of a rod-shaped DNA/ protein structure called nucleocapsid. The nucleocapsid may be found enveloped singly (SNPV) by a lipoprotein membrane envelop or multiple nucleocapsid (MNPV) are enveloped within each envelop. Between 20 to 200 virions are embedded in the polyhedral matrix depending upon the species. Ex. In *Ha*NPV (*Helicoverpa armigera* nuclear polyhedrosis virus) there are commonly up to 30 virions in each OB. These OBs are the infective stage of the viruses, designated to transmit the infection from insects to insects. The polyhedrin crystals help to protect the vulnerable virions from inactivation by environmental factors. Baculoviruses in this form are extremely stable and can retain infectivity for many years, if not exposed to UV light or high temperature (50°C). NPV can easily be seen under Light microscope, whereas other viruses can be seen under Electron microscope, and so are much more difficult to identify and isolate. NPV attracted the attention of pest control scientists interested in looking for an alternative to chemical pesticides, because they can cause highly infectious disease that kills the host insect within 5-7 days.

Mode of Action: The virus infects the insect in the form of a polyhedral inclusion body (PIB). Upon ingestion, these PIBs enter the mid gut of the insect and dissolves in the alkaline condition of the gut (pH 9-11) and release virions. These virions enter the cells of the mid gut and proceed to multiply in the nucleus. During initial infection new virions are produced and they spread disease/ infection to haemotocytes, tracheal cells, fat body cells, and hypodermis. In later stage of infection polyhedral are produced on which virions gets embedded. When insect gets killed/dies, it ruptures and releases up to 100 million PIBs.

Granulosis viruses: The genus *Granulovirus* (GV) is characterized by smaller, often ovoid occlusion bodies of 0.13×0. 50µ.m in size which normally contain a single ODV (Ackermann and Smirnoff 1983). GVs have been exclusively found in Lepidoptera.

Mode of action: The primary phase of infection is initiated by *ingestion of virus by larvae, followed by dissolution of granulin (matrix protein of occlusion* body). The cells endocytosis the virus released from the occlusion body, which is carried by actin filaments to the nucleus. At this point, the virus uncoats and releases its genome into the nuclear pores.

The genome is then incorporated into the host genome and is transcribed by host machinery.

Baculoviruses used for Insect Pest Control

***Helicoverpazea/ Helicoverpaarmigera* SNPVs.** The three important lepidopteran polyphagous insect pest's species, the tobacco budworm, *Helicoverpa. virescens*, the corn earworm, *H. zea*, and *H. armigera*, infest many economically important crops especially maize, cotton, sorghum, chickpea, and tomato through the world (Fitt, 1989). In early twentieth century, reports were published that heliothine larvae exhibited the classic "wilt" symptoms associated with nuclear polyhedrosis (Glaser and Chapman 1915; Stahler, 1939); thereafter, field tests indicated that heliothine NPVs had potential for controlling outbreaks of heliothine pests (Chamberlin and Dutky 1958; Tanada and Reiner 1962). The virus isolate SNPV from *H. zea* (HzSNPV) was isolated in 1961; and was the first baculovirus to be registered with the US Environmental Protection Agency for use as a pesticide for the management of *H. virescens* and *H. zea*. HzSNPV was the first baculovirus developed into a commercial product and sold under the trade names Viron H (Elcar)and Biotrol-VHZ (Ignoffo *et al*., 1965; Ignoffo, 1973, 1999). It is a relatively broad-range baculovirus and infects many species belonging to genera *Helicoverpa* and *Heliothis*. HzSNPV provided control of not only cotton bollworm, but also of pests belonging to these genera attacking soybean, sorghum, maize, tomato and beans. The production of this biopesticide was discontinued in the year 1982. During this period, the resistance to many synthetic chemical insecticides including pyrethroids, again revived the interest and dependence in HzSNPV; wasgot registered under the name GemStar™. The baculovirus HzSNPV is a product of choice for management of *Helicoverpa armigera* (Mettenmeyer, 2002).

Another viral biopesticide, HaSNPV is almost identical to HzSNPV and was registered in China as a pesticide in 1993 (Zhang *et al*., 1995). In India, it has been extensively used on cotton fields (Srinivasa *et al.*, 2008).

2. *Lymantria dispar* MNPV. Forest ecosystems tend to be more stable than agricultural systems, thus allows natural or applied baculoviruses to remain in the environment for long time and increasing the chance of natural epizootics by these agents. The temperate forests are often attacked and defoliated by Lepidopteran larvae (common species are: *Lymantriadispar, Lymantria monacha, Orgiya pseudotsugata* and *Panolis flammea*) and some Hymenoptera species (mainly *Neodiprion sertifer* and *Diprion pini*). The European gypsy moth, *L. dispar* outbreaks are characterized by very dense populations of larvae that defoliate entire trees. The subspecies of, *L. dispar*, are found throughout Europe, Russia, the northern two-thirds of China, the Korean peninsula, and Japan (Pogue and Schaefer 2007). The periodic epizootics and abundant larval mortality of gypsy moth larvae was attributed to a naturally occurring NPV (LdMNPV) (Reiff, 1911; Campbell and Podgwaite 1972). Studies on to control *L. dispar* with abaculovirus had begun as early as 1959 in the former Soviet Union (Lipa, 1998), while in the year 1963, US Forest Service initiated studies on the use of LdMNPV (Rollinson *et al*., 1965); which led to the development of the products Virin-ENShin the USSR and Gypchekin the USA (Lipa, 1998; Reardon *et al*., 2009), with an additional product, Disparvirus, developed from Gypchek for use in Canada (Zhang *et al*., 2010).

***Anticarsia gemmatalis* MNPV.** The velvetbean caterpillar, *Anticarsia gemmatalis,* is the most serious pest of soybean in Brazil and the south-eastern USA (Panizzi and Correˆa-Ferreira 1997). The use of *A. gemmatalis* MNPV (AgMNPV) in the control of velvet bean caterpillar *A. gemmatalis* has been the most successful control of the baculovirus used as the biological pesticide in Brazil (Moscardi, 1999). During 1977, Nucleopolyhedrosis viruses were isolated from *A. gemmatalis* in Brazil (Allen and Knell 1977; Carner and Turnipseed 1977). The field tests with one of the isolates of *A. gemmatalis* MNPV (AgMNPV) demonstrated to suppress *A. gemmatalis* populations in soybean. AgMNPV for use against *A. gemmatalis* infestations is produced in the field, on soybean farms, by private companies. Virus-killed larvae are harvested and AgMNPV is extracted and prepared as a wettable powder; the preparation doesn't have much cost and can be sold to the farmer for the price less than a chemical insecticide (Moscardi, 1999).

***Neodiprion* spp. NPVs.** The Pine sawfly, *Neodiprion* spp., larvaeare the most common defoliating pests of pine trees, *Pinus* spp., which causes growth loss and even plant mortality, especially when followed by secondary attack by bark and wood-boring beetles (Coleoptera: Buprestidae, Cerambycidae, Scolytidae,). The larvae of many sawflies species feed on pine trees. Among these, species of the genus *Neodiprion* are the most destructive defoliating pests of pine throughout North America and Europe (Coppel and Benjamin 1965; Haack and Mattson 1993). During severe infestation of sawflies, the larvae congregate and gregariously feed on pine needles, a trait which makes them an ideal target for control by baculoviruses. The Pine trees of all ages are susceptible to defoliation (Barnard and Dixon 1983; Coppel and Benjamin 1965). During severe pest infestation, the larvae in large numbers gregariously feed on pine needles, an ideal trait that makes them target at ease for control by baculoviruses.

The European pine sawfly, *N. sertifer*, is native to northern Europe and first appeared in North America in 1925. The Nucleopolyhedrovirus from European pine sawfly population in Sweden was first used successfully to control N. *sertifer* in Canada (Bird, 1953). In the field trials at USA, a commercial product Neochek-S registered therein reduced *N. sertifer* arval populations by up to 90 per cent and defoliation by 96per cent (Podgwaite *et al.*, 1984). An NPV from another pine sawfly species, *N. lecontei*, infesting red pine and jack pine plantations was identified and found to suppress *N. lecontei* populations (Bird, 1961). A commercial product, Lecontvirus to suppress *N. lecontei* infestations was registered in Canada in 1983.Another sawfly species, *N. abietis* a minor pest on balsam fir in the Canadian provinces of Newfound land

and Labrador until 1990s; thereafter a severe out break resulted in defoliation of young and prethinned balsam fir stands (Moreau, 2006). A baculovirus, NeabNPV isolated from *N. abietis* was amplified in the laboratory and field-tested by aerial application (Moreau, 2006). The virus application resulted in five-to-ten-fold decreases in sawfly population density. The commercial viral product got registered with the name Abietev in 2006; and is currently being applied for managing *N. abietis* outbreak in Newfoundland (Iqbal and MacLean 2010).

The first viral insecticide was the single- nucleocapsid-nucleopolyhedrosis virus of *Helicoverpa (Heliothis) zea* (HzSNPV) registered in 1971 (Ignoffo, 1973).

Some of the commercially available viruses for Lepidopteron control

Commercial Name	Producer	Viral species	Target pests
Granupom	AgrEvo	*Cydia pomonella* GV	Codling moth
Carposin	Agrichem	*Cydia pomonella* GV	Codling moth
VPN- 80	Agricola EI Sol	*Autographa californica* MNPV	Lepidopteran larvae
Polygen	Agroggen	*Anticarsia gemmatalis* MNPV	Velvetbean caterpillar, sugarcane borer
Capex 2	Andermatt Biocontrol	*Adoxophyes orana* GV	Summae fruit tortrix
Madex 3	Andermatt Biocontrol	*Cydia pomonella* GV	Codling moth
Ness-A	Applied Chemical	*Spodoptera exigua* MNPV	Beet armyworm
Ness-E	Applied Chemical	*Spodoptera exigua* MNPV	Beet armyworm
Leconteivirus	Canadian Forest Service	*Neodiprion sertifer /N. lecontel* MNPV	Sawfly larvae
Multigen	EMBRAPA	*Anticarsia gemmatalis* MNPV	Velvetbean caterpillar, sugarcane borer
Monisarmiovirus	Kemira	*Neodiprion sertifer/ N. lecontel* MNPV	Sawfly larvae
Virin- EKS	NPO Vector	*Mamestra brassicae* MNPV	Lepidopteran larvae
Virin- GYAP	NPO Vector	*Cydia pomonella* GV	Codling moth
Carpovirusine	NPP/Calliope	*Cydia pomonella* GV	Codling moth
Mamestrin	NPP/Calliope	*Mamestra brassicae* MNPV	Lepidopteran larvae
Virox	Oxford Virology	*Neodiprion sertifer/ N. lecontel* MNPV	Sawfly larvae
Elcar	Novartis	*Helicoverpa zea* SNPV	Heliothines
Cyd-X	Thermo Trilogy	*Cydia pomonella* GV	Codling moth
Gemstar	Thermo Trilogy	*Helicoverpa zea* SNPV	Heliothines
Gypcheck	U.S. Forest Service	*Lymantria dispar* MNPV	Gypsy moth
Spod-X	Thermo Trilogy	*Spodoptera exigua* MNPV	Beet armyworm

Based on Copping (1998), CPCR (1998), CDMS (1998), CEPA/DPR (1998)

FUTURE SCOPE

The use of synthetic insecticides will be continued due to limited information of alternative insect pest management practices. The effective public extension services, government policies, and farmer education are important to expand the use of viral insecticides as well as for further development in the production and use of these insecticides. In addition, introducing and evaluating commercial available baculoviruses bio-insecticides need to be practiced.

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