

# "Baculoviruses in Integrated Pest Management: A Focused Review on Structure, Classification, and Applications in Controlling Fall Armyworm, *Spodoptera frugiperda* "

## Abstract

Baculoviruses are crucial biological agents in integrated pest management (IPM), particularly for controlling lepidopteran pests in agriculture. These are highly specific, eco-friendly viruses characterized by rod-shaped nucleocapsids containing a protein-encased genome. The Baculoviridae family comprises four genera: Alphabaculovirus, Betabaculovirus, Gammabaculovirus, and Deltabaculovirus, each targeting specific insect orders. Nucleopolyhedrovirus (NPVs) and granuloviruses (GVs) are extensively used in pest management due to their high virulence and specificity, ensuring safety for non-target organisms. The infection process begins when insect larvae ingest viral occlusion bodies, leading to systemic infection and host death. Despite their narrow host range and slower action compared to chemical insecticides, baculoviruses are invaluable in sustainable agriculture. Their application has expanded beyond pest control to include gene therapy and vaccine production. This review examines the structure, classification, infection process, and current status of baculoviruses in controlling the fall armyworm, *Spodoptera frugiperda*, a significant agricultural pest. By exploring their potential as a cornerstone in biological pest management, we highlight the importance of baculoviruses in developing sustainable and environmentally friendly pest control strategies. The integration of baculoviruses into IPM programs offers a promising approach to reduce chemical pesticide use, preserve biodiversity, and enhance crop protection, ultimately contributing to more sustainable agricultural practices and food security.

**Key words:** Baculoviruses, Integrated Pest Management (IPM), Nucleopolyhedrovirus (NPVs), Fall armyworm, *Spodoptera frugiperda*, biological pest management

# 1. INTRODUCTION

## 1.1. Baculoviruses

Crop production globally faces significant challenges from pests and pathogens, particularly in tropical and subtropical monocultures. While synthetic chemical pesticides have been the primary pest management method, their use is increasingly problematic due to environmental harm, health risks, persistence, insect resistance, and high costs. These factors have led to growing farmer reluctance to use synthetic chemicals. In response, integrated pest management (IPM) has gained prominence, combining cultural, physiological, chemical, and biological controls to mitigate insecticide resistance and reduce environmental hazards [1-2]. IPM emphasizes eco-friendly, biodegradable alternatives for sustainable pest control, reduced reliance on synthetic chemicals, and preservation of beneficial organisms. This shift towards IPM represents a crucial step in developing more sustainable and environmentally responsible agricultural practices, addressing both pest management needs and broader ecological concerns.

Biological agents, including predators, parasitoids, and microbial pesticides (viruses, fungi, bacteria, and nematodes), have been utilized since the 1940s and 1950s for insect pest management. These methods are effective and safe for beneficial insects, making them a well-established approach in integrated pest management (IPM) strategies [3]. Viruses, particularly effective against larvae and certain insects like shrimp, mosquitoes, and sawflies, are non-infectious to mammals as they cannot replicate in mammalian cells [4-5]. Baculoviruses, named for their rod-shaped nucleocapsids ("baculum" meaning cane), exist in two forms: occlusion-derived viruses (ODVs) encapsulated in a crystalline protein matrix, and budded viruses (BVs) produced by diseased cells [6]. These nucleocapsids, typically 40-60 nm wide and 250-300 nm long, contain the viral DNA genome embedded within a protein capsid and associated with an arginine-rich basic protein [7]. The structure and composition of baculoviruses contribute to their effectiveness as biological control agents, offering a sustainable alternative to chemical pesticides in agriculture while preserving ecological balance.

## 1.2. Classification

The Baculoviridae family, comprising 66 species across four genera, is a prominent group of viruses widely used as biological insecticides [8-9]. These genera, *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus*, and *Deltabaculovirus* are target specific to insect orders: Lepidoptera (Alpha- and Betabaculoviruses), Hymenoptera (Gammabaculoviruses), and Diptera (Deltabaculoviruses) [6 & 10]. The family is further divided into two subfamilies: Eubaculovirinae, with virions enclosed in a protein matrix, and Nudibaculovirinae, lacking this protein covering. Within Eubaculovirinae, nuclear polyhedrosis viruses (NPVs) and granulosis viruses (GVs) are extensively used in pest management due to their high virulence and specificity to insect species. The term "virus," derived from Latin for poison, refers to disease-causing agents composed of nucleic acids encased in a protein shell (capsid). In biological pest control, NPVs are predominant, hence the term nucleopolyhedroviruses. Approximately 60% of Baculoviridae viruses are employed to combat pests threatening fiber and food crops, underscoring their significance in sustainable agriculture and integrated pest management strategies.

### 1.3. Structure of Baculoviruses

Baculoviruses are small, circular, double-stranded DNA viruses that infect a wide range of insects and arthropods. Their genetic material is encased in a protective protein shell called a polyhedron, which is further enveloped within occlusion bodies (OBs) for enhanced stability and longevity [6 & 11]. First identified in the crane fly (*Tipula paludosa*) and later in the spruce sawfly (*Gilpinia hercyniae*), baculoviruses include nucleopolyhedroviruses (NPVs) and granuloviruses. These viruses, along with polydnaviruses, ascoviruses, and reoviruses, are key pathogens in biological pest control. Beyond pest management, baculoviruses have applications in gene therapy and vaccine production [12-13]. Baculoviruses are particularly effective against Lepidoptera species, but also target insects from orders such as Hymenoptera, Diptera, Neuroptera, Coleoptera, Trichoptera, as well as some Crustacea and mites. The Baculoviridae family, comprising NPVs and granuloviruses, is widely used in pest management due to its eco-friendliness, effectiveness, stability, and environmental safety. NPVs, first recorded in 1913 as a filterable virus, infect hosts by attaching to them for extended periods, aided by occlusion bodies. These rod-shaped viruses multiply within hosts, ultimately causing death. In the 1940s, NPVs were introduced as biopesticides in crop fields. In India, NPVs have been documented in various insect species, including *Helicoverpa armigera*, *Spodoptera litura*, *S. exigua*, and several others. The

development and application of baculoviruses have been extensively reviewed globally [2]. Their ability to infect a broad range of insect pests, coupled with their specificity and environmental safety, makes baculoviruses invaluable tools in integrated pest management strategies. As research continues to advance, the potential applications of baculoviruses in agriculture, forestry, and biotechnology continue to expand, offering promising solutions for sustainable pest control and beyond.

#### **1.4. Characteristics of Nucleopolyhedrovirus**

Nucleopolyhedroviruses (NPVs) are widely used in integrated pest management due to their species-specific action, posing no harm to non-target organisms such as mammals, birds, and fish. They are safe for beneficial insects, including predators and pollinators, and are environmentally friendly, causing no damage to plants or humans. Additionally, NPVs offer effective natural pest control and are compatible with various integrated pest management strategies. These viruses can be produced at both farm and industrial scales, as highlighted [14]. However, certain factors limit their global use, including their narrow host range, slow speed in killing pests, and limited availability of samples. Although Baculoviruses have been used to enhance the toxicity of insecticides, there are still no improved or commercially available baculovirus-based insecticides [15].

#### **1.5. Host Range**

There are numerous viral families, but Baculoviruses specifically belong to the family Baculoviridae. These viruses have been isolated from insects in the orders Lepidoptera, Hymenoptera, and Diptera, with over 700 baculoviruses identified. The majority of these viruses have been isolated from Lepidoptera, with smaller numbers from Hymenoptera and Diptera. According to [16], more than 50 baculovirus-based products have been utilized globally for pest management. These viral products have been employed to control insect pests in forests, agriculture, and vegetable crops.

#### **1.6. Transmission**

Baculoviruses can be transmitted both vertically and horizontally from infected to healthy hosts, and these modes of transmission play a crucial role in the ecology of baculoviruses. Horizontal transmission refers to the spread of the virus from infected populations to healthy ones.

In this process, infected hosts die, and viral particles are released from their cadavers into the soil and onto foliage. Large quantities of occlusion bodies (OBs) are released from these dead cadavers, which are then ingested by susceptible hosts during feeding. Additionally, the host plants become contaminated with the feces of infected populations, serving as a significant source for further virus transmission.

### **1.7. Infectious process (larvae or adult etc.)**

A wide variety of pathogens, including parasites, predators, and parasitoids, have been observed to alter their host's behavior during infection [17]. Examples of this include *Toxoplasma*-infected rodents losing their instinctive aversion to cats, ants infected with lancet liver flukes climbing onto grass leaves, and Gordian worm-infected crickets and grasshoppers displaying altered behaviors. Baculoviruses, however, are unique in that they specifically infect the larval stages of insects. The infection typically occurs when the insect host ingests occlusion body (OB) contaminated plant material or soil. Baculoviruses produce two types of virions: occlusion-derived viruses (ODVs) and budded virions (BVs). ODVs facilitate horizontal transmission between insect hosts, while BVs are responsible for spreading the infection systemically within a single host. Once ingested, the OBs and food particles travel through the foregut and enter the larval midgut, where the infection process begins. Within approximately 10 minutes of entering the midgut, ODVs spread throughout the midgut, breaching the peritrophic membrane to infect midgut epithelial cells, leading to a systemic infection [18]. Lepidopteran larvae have highly alkaline midgut environments, with pH levels ranging from 10 to 11, and baculoviruses have evolved to exploit this alkaline microenvironment. The alkalinity of the midgut aids in dissolving OBs, releasing the occlusion-derived virions (ODVs) embedded within them into the midgut lumen. These ODVs are released within about 10 minutes of entering the midgut, where they subsequently breach the peritrophic membrane, invade the midgut epithelial cells, and establish an efficient systemic infection [18-19].

### **1.8. Symptoms**

Upon transmission into their hosts, viruses induce distinct behavioral changes in the larvae. Initially, larvae movement ceases, and feeding activity significantly decreases. Infected larvae often adhere to and hang from vegetation. Their bodies become swollen and exhibit a glossy appearance. A key distinction between Nucleopolyhedrovirus (NPVs) and Granuloviruses (GVs)

lies in their physical structures: NPVs form angular polyhedral inclusion bodies, whereas GVs are characterized by ellipsoidal shapes.

## 1.9. Life cycle

*Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) is one of the most extensively studied viruses within the family Baculoviridae. This virus was initially isolated from the alfalfa looper, a species belonging to the order Lepidoptera. The infection process begins when the larvae consume viral occlusion bodies (OBs). Upon ingestion, these OBs release occlusion-derived viruses (ODVs), which target the larval midgut, a process well-documented by various researchers. The ODVs attach specifically to the midgut epithelial cells, after which they enter the cytosol and nucleus of the cells, initiating viral gene expression. Following this, budded viruses (BVs) are produced and invade nearby tissues, such as the tracheal system and hemocytes of the insect. The AcMNPV then spreads throughout the insect's body, where it utilizes specific activating enzymes to break down insect tissues, facilitating the multiplication of the virus in the environment. This tissue degradation aids in the release of viral particles, enhancing their ability to infect new hosts.

## 2. Current Status of Viruses in the Control of FAW

Much of our current understanding of insect viruses comes from research focused on viruses that impact the mass rearing of economically significant insects, such as the silkworm *Bombyx mori*, the two-spotted cricket *Gryllus bimaculatus*, and the house cricket *Acheta domesticus*. Additionally, extensive study has been conducted on viruses with potential as biocontrol agents against major agricultural pests like the cotton bollworm *Helicoverpa zea* and forest defoliators such as the gypsy moth *Lymantria dispar*. These viruses include members of the ascovirus, baculovirus, and densovirus families [20-21]. Over the past decade, large-scale sequencing studies have led to the discovery of novel viruses in various lepidopteran species. In the following section, we will discuss the virus families associated with *S. frugiperda*, detailing their key biological characteristics, the symptoms they cause in susceptible insect stages, and their potential for use as biological control agents.

### 2.1. Ascoviruses

Ascoviruses (family Ascoviridae) are large, enveloped, double-stranded DNA (dsDNA) viruses with genome sizes ranging from 100 to 200 kilobase pairs (kbp) [22]. These viruses primarily infect species within the Noctuidae family of the order Lepidoptera, specifically targeting the larval stages [20-21]. Parasitoids serve as the natural vectors for ascoviruses in the field, facilitating their transmission. Infection by ascoviruses triggers the formation of virion-containing vesicles within the hemolymph of infected larvae, giving it a characteristic milky appearance—a hallmark of the disease [23-24]. The circulation of these virions and vesicles within the hemolymph enables mechanical transmission of the virus to healthy larvae or pupae through endoparasitic wasps during oviposition [23]. Ascoviruses cause chronic to fatal diseases in larvae, leading to stunted growth, molting difficulties, and eventual death. These infections are observed across all larval stages in natural populations of *S. frugiperda* [25]. The first reported case of ascovirus infection in natural FAW populations in the USA was documented [7]. Additionally, field surveys in Mexico identified two *S. frugiperda* larvae infected with ascoviruses, characterized by the presence of vesicles [26]. Despite their high virulence, ascoviruses have not been utilized in biological pest control due to significant limitations. Transmission by parasitoids poses a major challenge, as per os (oral) infection of larvae is rarely effective [27]. This inefficacy is attributed to the inability of ascoviruses to fully overcome the insect midgut barrier. A potential strategy to enhance the effectiveness of ascoviruses in biological control involves combining them with other insect pathogens capable of lysing the insect midgut and initiating infection. For example, mixtures of *Heliothis virescens* ascovirus isolates (HvAV-3h and HvAV-3j) with *Bacillus thuringiensis* kurstaki (Btk) were effective in lysing the midgut of lepidopteran species such as *Mythimna separata* and *Spodoptera litura*, resulting in increased mortality compared to the control group treated with Btk alone. However, this approach showed low mortality in *Helicoverpa armigera* and *S. frugiperda* [28-29]. The low mortality in *S. frugiperda* might be due to its low susceptibility to the Btk strain Cry1AC, or the inability of the *H. virescens* ascoviruses to infect *S. frugiperda*. Nonetheless, combining ascoviruses with other pathogens could potentially improve their per os infection efficiency, offering a promising alternative for pest control.

## **2.2. Baculoviruses**

Baculoviruses, members of the Baculoviridae family, are large, circular double-stranded DNA viruses with genomes ranging from 80 to 180 kbp. They primarily infect lepidopteran larvae, many

of agricultural importance [30]. The family comprises four genera: *Alphabaculovirus* and *Betabaculovirus* (infecting lepidopterans), *Gammabaculovirus* (infecting hymenopterans), and *Deltabaculovirus* (infecting a dipteran species [30-31]. These viruses exhibit a bi-phasic infection cycle with two virion types: occlusion-derived viruses (ODVs) and budded viruses (BVs). ODVs, encased in protective occlusion bodies (OBs), initiate midgut infection, while BVs facilitate systemic spread within the host. OBs provide environmental stability, allowing for long-term storage and use in biopesticide sprays [32]. Baculoviruses are morphologically distinguished as nucleopolyhedroviruses (NPVs) and granuloviruses (GVs), differing in their OB matrix proteins [33]. Their applications extend beyond pest control to biotechnology, serving as expression vectors and gene therapy delivery vehicles [34]. The co-evolution with hosts has resulted in a narrow host range, typically restricted to closely related species, making baculoviruses highly specific and environmentally safe alternatives to chemical pesticides [34]. Alphabaculoviruses infecting *Spodoptera* species, such as SfMNPV, SeMNPV, SpliNPV, and SpltNPV, exemplify this specificity. Baculovirus infections in larvae typically cause lethargy, pale discoloration, death, and often liquefaction [35-36]. Some baculoviruses manipulate host behavior, inducing hyperactivity and "tree-top" disease [37]. *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV) is the primary viral candidate for controlling the fall armyworm (FAW) globally. Various SfMNPV isolates have demonstrated high larval mortality in *S. frugiperda* [38]. Dead caterpillars serve as inoculum sources, promoting viral epizootics crucial for effective biological control [39]. Other baculoviruses, like *S. littoralis* nucleopolyhedrovirus (SpliNPV), have shown effectiveness against *S. frugiperda*, achieving up to 60% larval mortality in tests [40]. However, the inter-host effectiveness of non-specific isolates is often lower, emphasizing the importance of obtaining local SfMNPV or *S. frugiperda* granulovirus (SfGV) isolates for optimal control of local FAW populations [41-42]. The high specificity, environmental safety, and effectiveness of baculoviruses make them invaluable tools in integrated pest management (IPM) programs [43]. Their ability to cause lethal infections in target pests while sparing beneficial insects and posing no threat to humans or the environment positions baculoviruses as a sustainable alternative to chemical pesticides. As research continues to advance, the potential applications of baculoviruses in agriculture, forestry, and biotechnology expand, offering promising solutions for pest control and beyond. The ongoing study of baculovirus ecology, host interactions, and genetic diversity

contributes to the development of more effective biopesticides and biotechnological applications, further solidifying the role of these viruses in sustainable agriculture and scientific innovation.

### **3. SfMNPV: The Most Promising Viral Candidate for the Biological Control of FAW**

Over the past decades, numerous SfMNPV isolates have been obtained from dead *S. frugiperda* larvae collected in crop fields and pastures across the Americas [42] some of these isolates have been developed into commercial biopesticides. Following the first reports of FAW (fall armyworm) invasion in several countries in the Eastern Hemisphere, efforts have been directed towards collecting and characterizing effective natural enemies that may have co-invaded these new areas along with FAW. Baculoviruses, particularly SfMNPV and SfGV, are among these natural enemies, having been associated with FAW in its native range (Escribano *et al.*, 1999). However, knowledge about the occurrence of SfMNPV and SfGV in the invading FAW populations remains limited. Nonetheless, naturally occurring field isolates of SfMNPV have been discovered in newly invaded regions, including China, India [43] and Nigeria. For instance, initial characterization of an SfMNPV field isolate from China (SfHub) revealed two naturally occurring genotypes, SfHub-A and SfHub-E, which differ in their biological characteristics [44-45]. Commercial isolates of SfMNPV have been registered and successfully employed to control *S. frugiperda* in North and South America, and more recently in certain African and Asian countries [41]. To date, only two SfGV isolates have been isolated from *S. frugiperda* [47]. SfGVs are characterized by a relatively slow speed of kill, requiring up to 24 days to kill *S. frugiperda* larvae. The virulence of different SfMNPV and SfGV isolates, in terms of lethal concentration and speed of kill, varies widely. One major reason for these differences is the variation in specific genes within the genomes of the different isolates [35]. Beyond the core genes shared by most baculoviruses, variations due to insertions, deletions, and single nucleotide polymorphisms (SNPs) have been reported among different isolates [1]. Additionally, the virulence of SfMNPV isolates can also be influenced by the specific *S. frugiperda* strains present in the field. Bioassay experiments have shown varying levels of susceptibility in corn- and rice-strain larvae to the same SfMNPV isolates, with the corn strain exhibiting a broader range of susceptibility in terms of lethal concentration and speed of kill compared to the rice strain [38].

### **4. Specificity of SfMNPV**

Most baculoviruses are known to infect only one or a few closely related species, with some exceptions like *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Mamestra brassicae* MNPV (MbMNPV). These well-studied viruses are generalists with broad host ranges, capable of causing larval mortality in a wide array of insect species from different families [31]. In contrast, *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV) has a very narrow host range, primarily infecting only *S. frugiperda* larvae. Furthermore, similar to other baculoviruses, SfMNPV does not infect non-target organisms such as pollinators or other beneficial species. For instance, field-scale studies have shown that SfMNPV has no adverse effects on non-target and beneficial organisms, including predatory earwigs and beetles. This is in stark contrast to chemical pesticides like chlorpyrifos, which have been shown to harm natural enemies [47]. This suggests that when SfMNPV is introduced in new geographic regions, such as Africa or Asia, it is unlikely to have a significant impact on the local environment. Additionally, SfMNPV is highly compatible with integrated pest management (IPM) systems, successfully being used in combination with other control strategies such as spinosad [42], Bt foliar sprays, and transgenic Bt expression [42]. These characteristics make SfMNPV a highly suitable candidate for the biological control of *S. frugiperda*. Beyond inter-host specificity, intra-host population specificity is also a common phenomenon in many baculovirus-host interactions, including the SfMNPV-*S. frugiperda* complex. Previous studies have demonstrated that the susceptibility of *S. frugiperda* populations to SfMNPV isolates depends on the geographical origin of both the virus and the host population [12]. This intra-host specificity has been illustrated by the observation that SfMNPV isolates from local populations are often more effective against *S. frugiperda* populations from the same region. For example, the Colombian SfMNPV isolate (SfCol) was found to be 12 times more pathogenic to a Colombian *S. frugiperda* population than the Nicaraguan isolate (SfNIC). Similarly, *S. frugiperda* populations from Honduras showed greater susceptibility to neighboring isolates from Nicaragua (SfNIC) and the USA (Sf-US) than to a more geographically distant isolate from Argentina (Sf-Ar), which required 14 times the median lethal concentration to achieve the same mortality [42]. In the context of newly invaded regions, it is crucial to identify SfMNPV isolates associated with the local *S. frugiperda* populations to ensure effective control. This specificity highlights the importance of using locally adapted SfMNPV strains for the biological control of *S. frugiperda* in newly affected areas.

## **5. Conclusion**

This review underscores the significant role of baculoviruses in the integrated pest management (IPM) of *S. frugiperda*, commonly known as the fall armyworm. The detailed exploration of baculovirus structure, classification, and their mode of action highlights their unique attributes that make them suitable for biological control. Baculoviruses' high specificity and safety profile render them valuable tools in sustainable pest management strategies, offering a viable alternative to chemical pesticides. Despite the promising attributes of baculoviruses, several challenges remain, including their production, formulation, and environmental stability. Ongoing research and technological advancements are crucial for enhancing the efficacy, cost-effectiveness, and applicability of baculoviruses in diverse agricultural settings. Further studies are needed to address these challenges, optimize application methods, and integrate baculoviruses more effectively within IPM programs. In conclusion, the integration of baculoviruses into pest management strategies represents a forward-looking approach to addressing the global challenge of pest control. By leveraging the natural mechanisms of these viruses, it is possible to reduce dependency on chemical pesticides, mitigate environmental impacts, and achieve more sustainable agricultural practices.

### **Statements and Declarations**

#### **Data availability statement**

All the data have been provided in the main text of the manuscript.

#### **Ethics approval statement**

Not Applicable

#### **Consent to participate**

Not Applicable

#### **Consent to publish**

Not Applicable

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