Insect Viruses as Biocontrol Agents: Challenges and Opportunities

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Abstract Insect viruses were isolated from many insect pests from different families to represent a potential alternative for chemical pesticides. Viruses from families baculoviruses, cypoviruses, and densoviruses have been registered as biological control agents. Insect viruses are considered effective and environmental-friendly which may contribute to the achievement of sustainable agriculture goals through providing a suitable alternative to the chemical insecticides which have negative impacts on the environment and to the non-target organisms. However, the application of insect viruses as bio-control agents also have certain limitations. These include their slow action to their target, narrow host range, problems associated with the large-scale production and the development of insect host resistance against certain viruses. This chapter will discuss the challenges and the prospective use of insect viruses as biological control agents.

Keywords Insect viruses · Field application · Challenges and opportunities

1 Introduction

Insect viruses as biological control agent represent an important component in the integrated pest management (IPM) programs, mainly because it is specific and safe for the environment and compatible with the other integrated pest management components. Under certain situations Insect viruses cause epizootics in the field which provide added control of pests in nature. As insect viruses, i.e. baculoviruses are active in controlling early larval stage of lepidopteran insects, other IPM components such as chemical pesticides can be used to control late larval instars and entomopathogenic nematodes can target pupal stages under ground while mating disruption pheromone can be used to reduce mating in adult stages. Insect viruses were isolated from insect

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N. El-Wakeil et al. (eds.), *Cottage Industry of Biocontrol Agents and Their Applications*, https://doi.org/10.1007/978-3-030-33161-0_9

from different insect order such as Lepidoptera, Diptera, Orthoptera and Coleoptera [1–3].

This chapter will cover the following topics; the first part will focuss on the history of insect viruses as biocontrol agents for economic pests, followed by a discussion on the large scale production and commercialization of insect viruses with some examples. Thereafter, the emergence of resistance development and strain composition as challenges for insect viruses will be discussed with highlights on the recent reports in this field. Later the future and perspectives for the use of insect viruses as biocontrol agent will be summarized.

2 History for Insect Viruses as Biocontrol for Economic Pests

Pest management is an important part of both agricultural and forestry production system. In the past, as standard control measure chemicals were used. However, currently due to the negative impact of the chemical pesticides on the environment and the non-target organism and the increase in public concern to these effects, other methods, such as biological control approaches are becoming a viable option for the use of chemicals [4]. Biological control agents are living organisms that interfere with the productivity of other living organisms. In terms of biotechnology, biological control agents are used by human beings for the protection of resources that they want [5]. Biological control agents are composed of a wide range of organisms from vertebrates, insects, mites, plants, fungi, bacteria and viruses. Biological control programs have been successfully used to control noxious weeds, plant pathogens, invertebrate, and vertebrate pests [6]. The production, deployment, and the establishment of biological control agents are crucial parameters in determining the success of these agents for pest management. In addition, the latest improvement in genetic engineering has incorporated some biological control agents into the genomes of crops [7–9].

2.1 Biological Control of Insect, Mites and Nematode Pests

The reason why biological control has been used is to develop new, alternative, and environmentally friendly control agents. For instance, some insect viruses are promising candidates due to their inherent characteristics that appear among the promising candidates. Today, five groups of pathogens containing bacteria, viruses, protozoa, and nematodes are being used for control of insect pests. The microbial agents like viruses, bacteria, fungi and entomopathogenic nematodes were developed as biological control agents for a wide variety of arthropod pests [6, 10].

A variety of insect, mite and nematode pest control agents have been used as biocontrol. Reported examples are predators that feed on pests, parasitoids that lay eggs that grow in the pest and later kill it, parasites that weaken the pest and pathogens that infect and kill their pest host [11]. The most common form of the biological control method is the classical one whereby exotic natural enemies are introduced to control the exotic pests [12]. Three hundred million hectares of land (8% of agricultural land) is used for traditional biological control. During the last 120 years, nearly 2000 species of exotic arthropod agents have been announced as arthropod pests in 196 countries or islands. More than 170 species are commercially available for pest control [13]. For instance, *Rodolia cardinalis*, the vedalia ladybird beetle is used as a biological control agent against the insect pest cottony cushion scale, on commercial citrus in California which was one of the first large-scale successes. Since then, the *Vedalia* beetle has now been controlling cotton cushion scale for over 100 years in more than 50 countries. Insect-specific viruses such as baculoviruses, have been extracted, assessed and mass-produced for use against codling moth in apple orchards. So far, the best outcome for management of codling moth occurs when virus applications are combined with mating disruption. Parasitic wasp (*Hymenoptera: Encyrtidae*) is also used as a biocontrol agent in orchards [14].

2.2 Biocontrol of Vertebrate Pests

Biological control technology has also been used against vertebrate pests (rabbits, rats, etc.) and veterinary and medical pests (nuisances, parasites, and diseases). For instance, the myxoma virus (*Leporipoxvirus, Poxviridae*) which causes myxomatosis, was introduced into Australia and spread rapidly throughout and reduced the rabbit population around 75–95% in 1950. In Australia, mosquitos were the main carriers for spreading the virus among rabbits. Finally, the rabbits developed immunity to the virus with virulence decreasing to 50%. [15]. In 1995, a new virus, rabbit hemorrhagic disease (RHD) was established on the mainland of Australia, and rat mortality again decreased to 50–90% specifically in the dry regions. Flies, mosquitos and rabbit fleas were the main vectors. In the temperate areas, rabbit populations returned to pre-RHD levels [15]. The use of viral pathogens led to a dramatic decline at the beginning, however vertebrate pests eventually developed varying degrees of genetic resistance. In addition, the use of microbes has been directed against human beings in times of war through direct application of the agents or using intermediary vectors such as insects to spread disease [16].

2.3 Viruses as Biological Control Agents

Insect viruses are obligate disease-causing organisms that could solely reproduce within a host insect. In the frame of integrated pest management program, viruses are the agents that provide the most safe, efficient and sustainable control method of insect pests. Some of the insect viruses are registered and produced as commercial products. However other viruses are naturally occurring, and they might even initiate an outbreak without any trigger or by unknown triggers especially for fruit fly pests [17]. Virus diseases were reported from more than 800 species of insects and mites. The family baculoviridae composes the most common and widely studied group of pathogenic viruses of insects. Baculoviruses are invertebrate-specific pathogens that in some cases were developed as biopesticides for the control of insects particularly species of the Lepidoptera [18].

Baculoviruses and cypoviruses (cytoplasmic polyhedrosis virus groups) have some biological properties which lead to their successful use as microbial control agents in integrated pest management programs such as, having protective occlusion bodies (polyhedra) which protect and increase the sustainability of these viruses. Baculoviruses generate two different phenotypes in their replicative cycle: the occlusion-derived virus (ODV) needed to spread the infection between larvae, and the budded virus (BV) needed for the dissemination of the infection within the host [19]. They are restricted to a single type of insect and extremely unambiguous for their host range systems. Numerous virus types have been determined among the members of arthropod species.

Research on insect virus in China recorded that more than 200 insect viruses isolates from several virus families like Baculoviridae, Reoviridae, Densovirinae, and Entomopoxvirinae can cause epizootics in natural populations of insects [7]. Some viruses are registered and commercially available, but their selectivity and small potential market might restrict their industrial interest. However, improvements in virus production, formulation and a better understanding of virus epizootiology should shed light to their enhanced role for this group of insect pathogens to become largely used as biological control. As microbial control agents, baculoviruses (nucleopolyhedroviruses and granuloviruses) are the most widely preferred and utilized [2, 20, 21]. The viruses are usually transmitted *per os* and gain access to host tissues via the midgut where the OBs that surrounded the virus rods are softened. In addition to baculoviruses, viruses from crypoviruses and densoviruses are registered as biological control agents in China. Recently nudiviruses such as the virus of *Oryctes rhinoceros* L. has been reported as the most successfully used non-occluded virus. Viruses comprise most of the host-specific entomopathogens, but their main drawbacks are the requirements for invivo production and their sensitivity to ultra-violet degradation.

2.4 Virus Discovery and Detection

Invertebrate pathology is a recently organized discipline, but its roots can be traced to ancient history concerning solutions for preventing disease in honey bees and silkworms [22, 23]. The use of microorganisms for control of insect pests was suggested by Basi, Louis Pasteur, and Elie Metchnikoff [22, 24]. The use of fungi as microbial control agents was suggested by several researchers in the late 19th century. It was not until the development of the bacterium *Bacillus thuringiensis Berliner* that the use of microbes for the control of insects became extensive. Today, plenty of entomopathogens are used for the control of invertebrate pests in a glass house, row crops orchards, ornamentals, range turf, lawn, stored products, forestry, for the abatement of pest and vector insects of veterinary and medical importance [25–27]. Similar to other natural enemies, insect pathogens could be used as a natural control way of the targeted populations. Most of the epizootics are naturally occurring due to viral and fungal pathogens that are responsible for spectacular crashes of insect pest populations [28].

Virus infection begins in the insect's digestive system but spreads throughout the whole body of the host in fatal infections. The body tissues of virus-killed insects are almost completely converted into virus particles. The digestive system is among the last internal organ system to be destroyed, and therefore the infected insects usually continue to feed until they die. Infected insects appear normal until just before their death when they tend to darken in color and move slowly. They often develop slow than uninfected individuals [29–31]. Most virus-infected insects die while attached to the plant on which they feed. Virus-killed insects break open and disseminate virus particles into the environment. These virus particles can infect new insect hosts. Because of the damage of the inner tissues, dead insects often have a "melted" look. The contents of a dead insect can range from milky-white to dark brown or black. While natural virus outbreaks tend to be localized, virus particles can be spread by the movement of infected insects, or the predators such as other insects or birds that come into contact with the infected insects, or non-biological factors like water run-off, rain-splash or air-borne soil particles. Many virus-infected insects also climb to higher positions on their host plant before they die, which maximizes the spread of virus particles after the insect dies and disintegrates. The number of virus infection cycles within a growing season depends heavily on the insect's life cycle. Insect pests with multiple generations per season or longer life cycles can be more heavily impacted by virus outbreaks since there is a greater opportunity for multiple virus infection cycles within a growing season [32].

2.5 Virus Group Used as Biological Control

The major groups of insect viruses that might be used as biocontrol agents for economic pests are presented in the table below (Table 1).

At least 11 groups of viruses, including the Baculoviridae, Reoviridae, Poxviridae, and the Iridoviridae, are known to cause diseases in insects [33]. Baculoviridae are confined to arthropods; replicating in Lepidoptera (butterflies and moths), Hymenoptera (wasp), Diptera (flies), Coleoptera (beetles), Neuroptera (lacewings), Arachnida (spiders) and Crustacea (prawns and shrimps) [34]. In other groups of viruses, some members are also pathogenic to vertebrates and/or plants [35]. Only

Table 1 Major groups of entomopathogenic viruses

Virus groups	Nucleic acids	Morphology of virus particle	Presence of inclusion body	Host range in arthropods	Related virus groups
Baculoviridae (a) Nuclear polyhedrosis virus (b) Granulosis virus	Circular dsDNA	Bacilliform	+	Lepidoptera, diptera, hymenoptera, coleoptera, neuroptera, crustacea, mites	No viruses with similar morphology found in vertebrates or plants
Nudiviridae	Circular dsDNA	Bacilliform	–	Coleoptera	No viruses with similar morphology found in vertebrates or plants
Reoviridae (cytoplasmic polyhedrosis viruses, cypoviruses)	Segmented dsRNA	Isometric	+	Diptera, lepidoptera, hymenoptera, crustacea	Other members of Reoviridae infect vertebrate and plant hosts
Poxviridae – Entomopoxviruses	Linear dsDNA	Brick-shaped	+	Lepidoptera, diptera, coleoptera, orthoptera	Other members of Poxviridae infect vertebrate hosts
Iridoviridae (Iridoviruses)	Linear dsDNA	Isometric	+	Diptera, lepidoptera, coleoptera, hymenoptera, hemiptera	Other proposed members of Iridoviridae infect vertebrate hosts
Parvoviridae (densoviruses)	Linear dsDNA	Isometric	–	Diptera, lepidoptera, orthoptera	Other members of parvoviridae infect vertebrate hosts
Picornaviridae Small RNA viruses (many groups)	ssRNA	Isometric	–	Diptera, lepidoptera, hymenoptera, hemiptera orthoptera, coleoptera hymenoptera, hemiptera	Viruses with similar morphological and bio-chemical characteristics infect vertebrate and plant hosts

Adapted from [33]

one insect-pathogenic virus Nodamura virus (family: Nodaviridae) is known to infect a vertebrate and an insect and is transmissible to suckling mice by *Aedes aegypti* [36]. Most of the research and developments of viral bioinsecticides activities are focused on Baculoviridae due to its safety and the large number of viruses isolated from economic pests. Baculoviruses include over 1690 viruses that have been recorded from more than 1100 species of insects and mites [37, 38]. Of these, three families (Baculoviridae, Polydnaviridae, Ascoviridae) are specific to insects and related arthropods. The baculoviruses are the most widely exploited virus group for biocontrol: they are very different from viruses that infect vertebrates and are considered safe to be used as biopesticides.

The mode of pathogenesis and replication of entomopathogenic viruses vary according to the family, but the infection nearly always occurs through ingestion by the host. Virions then bind to receptors in the gut and penetrate epithelial cells and initiate infection. In baculoviruses, the infection often spreads to the hemocoel and then to essential organs and tissues, particularly fat bodies. Acute infections lead to host death in 5–14 days. There are two genera of baculoviruses: nucleopolyhedroviruses (NPV) and granuloviruses (GV). The host range of baculoviruses is restricted to the order, and usually the family of origin of the host. The commercial production for baculoviruses as biopesticides are considered to present a minimum risk to people and wildlife. Mass production of baculoviruses can only be done in vivo but it is economically viable only for larger hosts such as Lepidoptera, and the formulation and application are straightforward [37]. Currently, there are approximately 16 biopesticides based on baculoviruses presented for use or under development. The majority of these products are targeted against *Lepidoptera*. For example, codling moth granulovirus, CpGV (*Cydia pomonella* Granulovirus) is an effective biopesticide of codling moth caterpillar, pests of apples [39].

The baculovirus virions are enveloped rod-shaped nucleocapsids comprising circular, supercoiled, double-stranded DNA. The virions of GVs are individually occluded in a protein matrix (granulin). In the NPVs single enveloped (SNPV) or multiple enveloped (MNPV) virions are occluded in a protein matrix (polyhedrin). The occlusion bodies or polyhedra are dissolved in the alkaline environment of the host's insect midgut after being absorbed by the host. The free virions enter the gut epithelial cells and replicate in the nuclei. Non-occluded virus particles that are budded from the gut cells into the hemocoel attack other tissues (fat, tracheal matrix, hypodermis, etc.) within the host. Virus particles that are occluded within polyhedra are generally the infective inoculum for subsequent hosts. Part of baculovirus virions transmission may be facilitated by predators and ovipositing parasitoids via mechanical transmission [28, 35].

As with other biocontrol agents, there are three basic strategies for the use of entomopathogenic viruses as microbial agents, which include inoculation, augmentation, and conservation. In most crops the use of viral pathogens of insects is intentive and does not use their full epizootic potential, but take the advantage of their virulence and specificity [33]. Baculoviruses registered for use or under development for insect pest control are presented in Table 2.

Table 2 Baculoviruses registered for use or under development for control of insect pests in agroecosystems, stored products, and forestry

Virus	Target insect	Crop	Commercial product	Country	Reference
Anticarsia gemmatalis NPV	The velvet bean caterpillar	Soybeans		Brazil, Paraguay	[52]
Cydia pomonella (CpGV)	The codling moth	Pear and apple	Cyd-X and VirosoftCP4 in North America and in Europe include Carpovirusine™ (France), Madex™ and Granupom™ (Switzerland), Granusal™ (Germany), and Virin-CyAP	North America and Europe	[53]
Helicoverpa armigera NPV	The cotton bollworm, corn earworm, tobacco budworm budworms, corn earworm	Cotton, *Row Crops*	Gemstar LC (Certis USA)	China, USA	[54]
Mamestra brassicae NPV	Cabbage moth	Vegetables		China	
Autographa californica NPV	Beet armyworm Alfalfa looper and several other lepidopteran species	Vegetables		China	[55]
Spodoptera exigua NPV	Beet armyworm	Vegetables	Spod-X LC (Certis USA)	China	
Spodoptera litura NPV	Cotton leafworm	Vegetables		China	

(continued)

Table 2 (continued)

Virus	Target insect	Crop	Commercial product	Country	Reference
Plutella xylostella GV	Diamondback moth	Vegetables		China	
Trichoplusia ni NPV	Cabbage looper	Vegetables			[56]

Adapted from [7]

3 Large Scale Production and Commercialization of Insect Viruses

Baculoviruses have been isolated from more than 500 host species, with most of them from nucleopolyhedroviruses; 456 in Lepidoptera, 30 in Hymenoptera and 27 viruses in Diptera. Granuloviruses are specific to Lepidoptera with 148 reported cases [39]. The use of insect viruses as biological control agents for insect pests was mainly focused on baculoviruses, one cypovirus (CPV) and one densovirus which have been registered as commercial bioinsecticides in China [7, 8].

There have been many reviews on the development of baculoviruses that are currently registered and used to control insects on large scale. Some of these viruses are listed in Table 1. Based on the treated area, three viruses are the most used ones and are reviewed by Rohrman [40]. In brief, the NPV of the velvet bean caterpillar *Anticarsia gemmatalis* isolated in 1977 in Brazil became the most successful example of a virus used as a biological pesticide with more than 2 million ha treated area per year in 2006, providing effective and safe control of larvae of the key crop defoliator of soybean fields [21, 41, 42]. The second most widely used viral pesticide is the granulovirus of the codling moth, *Cydia pomonella* (CpGV) in many countries in North America and Europe since 2000 for the control of the insect on pear and apple crops. The virus was isolated in Mexico in 1963 [43] and it is currently produced under many commercial products in different countries (see Table 1). The third most widely used viral biopesticide is the NPV of the cotton bollworm, *Helicoverpa armigera* isolated in china in 1976, and was commercially produced as biopesticide in 1993 to control this pest. In 2005, the production of this biopesticide was around 1600 tons of infected insect [7, 8, 40]. In addition to these examples many other viral products are currently being used as biological control, some of them are list in Table 1.

3.1 Method for the Large-Scale Production of Viral Pesticides

Insect viruses are obligate pathogens and so far, cannot be produced outside the host cells. Therefore, the most common method for the virus production is in vivo through infecting the host insect in the production facility or in field by using the host insect cell line for the virus production.

3.1.1 Virus Production by Infecting Insect Host in Production Facilities

Most of the viral biopesticides are produced through infection the insect host in production facilities such as the production of CpGV and HearMNPV. This method requires the establishment of large-scale rearing facility of the insect host and infection of the susceptible stage (larvae) at relatively later stage with optimized virus concentration and ensuring the production of the highest viral production. The maintenance of mass production facility of the insect host requires the implementation strict hygiene measures to avoid accidental pathogen infection in the insect colony. In most cases, two separate production lines should be maintained, healthy colony and viral production line. This method is mainly suitable for insect host with biology enabling the economic mass-rearing.

3.1.2 Virus Production by Collecting Infected Larvae from Treated Filed

In some cases where the mass rearing of the host is not economically feasible or the production of the virus through infecting the host is not enough; collecting the infected larvae from the field remain the suitable option. This method was used in Brazil to produce the NPV of the velvet bean caterpillar, *Anticarsia gemmatalis* when the laboratory production was not found to be economically viable. In this case the virus production was carried out in farmers' fields. Plots of soybeans that were naturally infested with *A. gemmatalis* were sprayed with virus and then the dead larvae were collected 8–10 days after virus application. In this method, the major problem is that the virus production will depend on the natural host, prevalence and consequently the cost of the collection. In Brazil, individuals were able to collect about 1.8 kg of larvae/day at a cost in the mid-1990s of about $15. However, the viral production varied in the 1990s from enough virus to treat 650,000 to 1.7 million ha/year. By 1999, the production of virus was not sufficient to meet the demand [40].

3.1.3 Virus Production in Insect Host Cell Line

Due to the various challenges associated with the production of baculoviruses as biopesticides in infected larvae system such as the high cost and the unexpected failure in the healthy colony, the production of the viruses under fully controlled system such as insect cell line became an option to meet the market requirement. The cell culture growing in suspension that display a doubling time of 24 h or less and can be scaled up to 10,000 L in airlift or stirred tank bioreactors represent a feasible option for the virus large scale production. In some cases, the viral host is not feasible for mass-rearing and there is no cell line available for production of the virus and therefore an alternative host cell line such as the production of the nonoccluded *Oryctes* nudivirus, OrNV, using an adherent coleopteran cell line are used [44].

There are several cell culture systems that have received attention for their ability to produce insect viruses in large scale such as the *Heliothes zea* (HzAMI) cell line to produce *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV), the *Spodoptera frugiperda* SF9 cell line to produce *S. frugiperda* multicapsid nucleopolyhedrovirus SfMNPV and the *Anticarsia gemmatalis* cell line to produce *Anticarsia gemmatalis* nucleopolyhedrovirus, AgMNPV [45–51]. However, managing problems related to stability of the virus strains in culture, enhancing virus yields per cell through an understanding of how the host cell responds to the infecting virus, and the development of chemically defined media and feeds for the desired production systems remains the main challenges for cell culture-based production of insecticidal viruses [44]. In the above-mentioned examples, the virus production remains an option to overcome the production cost problem. However, in the case of the production of the non-occluded *Oryctes* nudivirus (OrNV) in the slow growing adherent coleopteran cell line DSIR-HA-1179, it is mandatory to overcome limitations associated with production of the virus in infected larvae. This system is well described by Reid et al. [44].

4 Resistance Development and Strain Composition as Challenges for Insect Viruses

As stated previously in this chapter, baculoviruses are among the insect viruses that are regarded safe for use as biological control agents due to their host-specificity and therefore, in this chapter they have been used as examples. Their efficient use as biological control agents is determined by the interaction of the virus and the insect-host in the field. The most important requirement for a successful control is the correct virus dosage to mortality ratio of an insect pest. This ratio changes with time due to circumstances in the field that lead to insect resistance to baculoviruses thus affecting the efficiency of the virus as a control measure. Different insect populations present variable responses to particular virus infections as well as a considerable variability in individual insect responses to different virus dosage. There are three main factors that determine the response of an insect to virus infection which contribute to the resistance development. These are categorised into the developmental, environmental and genetic, with each of them influencing the expression of insect resistance to baculoviruses. The developmental and environmental factors mostly lead to short-term resistance while the genetic factors mainly affect the long-term expression of resistance. This section of the chapter will discuss the mechanisms of resistance development in insects to baculoviruses and how the interplay between these factors influence the application of baculoviruses as biocontrol agents.

4.1 Mechanisms of Resistance Development

4.1.1 Developmental Factors

These are factors related to the growth and maturation of an insect through different stages. The relationship between the age of an insect and its response to virus infections has been reported in many insects such as *Lymantria dispar* and *Heliothis virescens* resistance to baculovirus [57–59]. It has been demonstrated that the resistance of an insect increases with larval age mainly due to the ability of an insect host to renew midgut cells during larval development which allows the elimination of the infected cells [60]. Further, as larval weight is related to developmental stage, the increase in resistance is reported to be directly proportional to the weight of the larvae. The increase in the larval weight reduces the surface-volume ratio of the midgut of the larvae and therefore increase the probability of the virus particles to pass through the midgut without attaching to the susceptible epithelial cells [60, 61]. Levy et al. [62] demonstrated increased resistant of *Anticarsia gemmatalis* larvae to AgMNPV which was correlated to increasing thickness of the peritrophic membrane (PM) which would protect the infection of the midgut cells. However, the presence of stilbene-derived optical brighteners which interfere with the synthesis of chitin, (a component of the PM), is known to reduce developmental resistance [63, 64]. Other physiological changes related to larval age/weight such as increase in gut PH with age are also known to affect an insect's resistance to virus infection. Therefore, the highest level of resistance is observed in the final-instar larvae just prior to pupation which confirms the age-related resistance. Moreover, this has also been associated with the shift in the balance of juvenile and molting hormones at the late instar stage [65]. This resistance justifies the importance of early treatments when using baculoviruses for pest control.

4.1.2 Environmental Factors

This involves the external influences that may trigger the insect defense mechanisms and therefore lead to the expression of resistance. Environmental factors affect the relationship between the virus and its insect-host, by acting either directly to the virus and affect its prevalence in the field or directly on the insect and alter its response to virus infection. This was initially evidenced in silk moth *Bombyx mori*, whereby high viral infections were observed in *B. mori* larvae in autumn than in spring [66]. These differences were associated with the quality of the mulberry leaves fed by the larvae due to changes in the sucrose, protein and cellulose levels in the leaves. For instance, protein levels directly affect the antiviral and protease activity of the larval digestive fluids while sucrose deficiency increases the uptake to the virus by the midgut epithelial cells and hence increase their susceptibility to virus infection. Physical factors such as temperature and light have also been shown to influence insect resistance [61]. For example, high temperature treatments increase resistance levels of late-instar larvae to virus infections. In the case of light, the level of resistance increases with the duration of larvae exposure to light, as reported for *B. mori* larvae that were reared in constant darkness were found to be more susceptible to virus infection than those reared in light [66]. This suggests that insects exposed to abnormal environmental conditions are more susceptible to virus infections.

4.1.3 Genetic Factors

These are factors that influence gene expressions, therefore regulating the immune defense mechanisms of an insect to virus infection. There are several genetic factors that contribute to the variability in resistance development in insects. First, most of the baculoviruses used as biological control agents are endemic since they have coevolved with their hosts. Due to this virus-host coevolution, particular host genes may become fixed in a certain population to offer resistance to virus infections. Differences in susceptibility to baculovirus infections have been reported in geographically distinct populations of the same species. Other studies investigating different baculovirus strains concluded that the resistance maybe influenced by a complex genetic mechanism or by single autosomal genes (either dominant or recessive alleles). For instance, resistance of *Cydia pomonella* (codling moth) to *C. pomonella* granulovirus (CpGV) is restricted specifically to the CpGV-M genotype and not to other isolates such as CpGV-I12 or CpGV-R5 [67, 68]. This resistance difference between the isolates has been associated with a viral gene, pe38, which for instance allows CpGV-R5 to replicate and not CpGV-M in resistant larvae [69]. In addition, the heredity of CpGV-M resistance is described as monogenic and sex-linked, since heterozygous and homozygous males showed different levels of resistance which could be as a result of a gene dosage effect [67, 70, 71]. Second, studies on selection of resistance genes within a population have shown that some individual insects possess a resistance gene, however, this requires several generations under selection pressure from the virus. For example, after selection against AgMNPV, *Anticarsia gemmatalis* reverted to the original levels after a few generations without virus treatment [72, 73]. On the other hand, *C. pomonella* resistance to CpGV-M remained stable for over 30 generations without virus treatment [74]. The existence of viruses in latent forms has also been shown to contribute to resistance, since they can be activated by several stressors including other viruses or virus strains which are genetically variable and subject to selection for changes in virulence.

4.2 Interplay Between Developmental, Environmental and Genetic Factors to Resistance Development

The interaction between these three major factors contribute to resistance development by affecting the insect's defense mechanisms to viral attack. First, resistance to virus infection can develop at any stage of virus infection following the initial viral attack. The most common route of baculoviruses entry is *per os* through the midgut lumen followed by the attachment, entry and establishment of infection in the midgut columnar cells and later the entry of virus particles into hemocoel to initiate secondary infections. Although there are several defense mechanisms that can act at any stage of the infection process, majority of the defences are activated during the initial viral invasion which prevents successful entry of the virus to the susceptible midgut cells. This contributes to resistance development to peroral infection and can greatly be affected by the developmental factors. For example, one of the insect's defense mechanism against viral invasion involves discharge of infected midgut cells into the gut lumen at each larval molting stage and their replacement with new cells. In addition, this type of defense (discharge and regeneration of columnar cells) can be influenced by genetic factors since in some insect populations, such as *B. mori* infected with cytoplasmic polyhedrosis virus (CPV), the regenerated columnar cells became re-infected while, *Hyphantria cunea* larvae infected with the BmCPV, the regenerated cells became immune to subsequent infection [60, 75].

On the other hand, environmental factors can interact with both genetic and developmental factors and influence the initial defense mechanism; light can influence the cell composition of the midgut epithelium, temperature can induce cellular discharge while both light and nutrient levels can affect the synthesis of antiviral agents. Temperature and photoperiod can affect the metabolic rates of an insect via hormone production and affect the rate of virus infection. For example, in two populations of *Pieris brassicae* larvae subjected to the same nutritional stress presented difference in response to GV infection; one population showed increase in susceptibility while the other showed resistance. This shows a correlation between the three major factors affecting the expression of resistance in that, as an insect develops a high virus dose is required to initiate a lethal infection, however, there are defense mechanisms that exist to counterattack the infection which are genetically (host or viral) influenced and may be subject to environmental stimuli [76].

The effects of the environmental factors in the field application appear to cause small changes in response since they involve aspects of nutrition or climate that are uncontrollable. Genetic factors are associated with the developmental factors which mainly cause age-related resistance. The genetic factors are subject to selection which can affect long term procedures for baculovirus application since a relatively small change in response could alter the cost effectiveness of viral control. For instance, frequent application of baculoviruses at high dose can increase the risk of resistance build-up by destabilizing any coevolved host-virus balance and therefore promote spread of resistance. As stated earlier, the age-related (developmental) resistance likely develops into resistant individuals than into susceptible ones. While the resistance may develop in an early instar larva, the selection pressure is usually stronger if late instar larvae were exposed to virus. Hence, in many cases the most cost-effective method of baculoviruses application in the field is against the early larval instars of the pests which reduces the risk of selection for increased resistance as well as early protection to the crops [60, 65, 77].

5 Future Perspectives for the Use of Insect Viruses as Biocontrol Agent

The specificity and the production of secondary inoculum make baculoviruses and other insect viruses attractive alternatives to chemicals insecticides and ideal components of Insect Pest Management systems due to their lack of unwanted negative effects on nontargeted beneficial insects including other biological control organisms or any negative impact on the environment and the ecosystems [78–80]. In addition, the use of insect viruses as bioinsecticides is compatible with many other components of biological control agents such as insect predators and parasitoids or other insect pathogens such as entomopathogenic bacteria or fungi in the frame of integrated pest-management. In addition, the fact that insect viruses are unable to infect mammals, including humans, makes them very safe to handle and attractive candidates as alterative biopesticides to avoid the use of the harmful chemical pesticides. However, despite the above-mentioned advantages, insect virus biopesticides products still represent a small fraction of the insect pesticides market, mainly due to certain limitations such as the narrow host range, the slow killing and loss of effect due to the exposure to UV light in the sun and recently the development of resistance in the host insect against the used viruses. Therefore, the future for the continuous use of the insect viruses will depend on the success to overcome these limitations.

The narrow host range can be faced through the use of biopesticide composed of virus mixture to increase the range of effectiveness of one product that can be used against several pests which will increase the market value of such product. The development of formulation which include protectant materials against UV could increase the sustainability of the viral product that can tolerate the UV effect and therefore increase the virus persistence [81, 82]. The use of recombinant bocaviruses that include the deletion of virus genes that delay the virus killing (e.g. the deletion of the ecdysteroid UDP-glucosyltransferase (*egt*) gene) or the expression of toxins that accelerate the killing effect has been developed for some viruses. However there are several challenges facing the large-scale production of these viruses as fast killing of the host affect negatively the amount of produced virus from infected host [7–9]. Finally, the use of the correct virus (or a mixture of virus) strains in the biopesticides to overcome the development of resistant against the virus in the host population might help to face the resistance challenges [68]. The success in facing the abomination limitation will shape the future use of insect viruses as biopesticides to control the major insect pests.

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