Chapter 12
Entomopathogenic Viruses

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12.1 Introduction

Brazil is essentially a tropical country with continental dimensions and several agricultural frontiers. The most recent MATOPIBA border includes part of the states of **Ma**ranhão, **To**cantins, **Pi**auí, and **Ba**hia. The average annual temperature in these regions is always favorable to the appearance of insect pests in many different crops. Faced with the current Brazilian landscape, these regions reach up to three annual crops with a supply of "green" food for insect pests from planting to harvest due to the overlapping of crops throughout the year. This factor is called "green bridge." As large areas of agricultural frontiers mainly plant soybeans, cotton, maize, and beans, insect pests such as caterpillars migrate easily between crops, for example, the soybean looper, *Chrysodeixis* sp., which is also a major cotton pest. Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Noctuidae), is one of the most important corn pests, but it is also a pest of soy, cotton, and beans. The same happens with the cotton bollworm *Helicoverpa armigera* (Hübner) (Noctuidae) that attacks corn, soybean, and cotton. The control of these agricultural pests is essentially done with the indiscriminate use of chemical insecticides, which has generated environmental pollution throughout the planet, besides causing the intoxication of applicators, rivers, and springs and the contamination of the final product to be sold in the market, both *in natura* and in processed ones. Among the biological control agents, viruses, especially those from the baculovirus group, are a viable alternative for pest control of agricultural importance and are a fundamental tool within the context of integrated pest management (IPM). Baculoviruses are host-specific restricted to arthropods (Organisation for Economic Co-operation and Development 2002). So far, no cases of pathogenicity of a baculovirus to a vertebrate have been reported (Krieg et al. 1980; Entwistle 1983).

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More than 17 families or groups of invertebrate viruses have been recognized as pathogenic to insects, of which baculoviruses have been the most documented (Harrison et al. 2016). Baculoviruses are genetically and morphologically distinct from other invertebrate virus families and are the largest group of insect-attacking viruses. According to Miller (1997) and Eberle et al. (2012a, b), the number of baculoviruses described to date, over 600, is from the family Baculoviridae infecting Lepidoptera (butterflies and moths), Hymenoptera (sawflies), and Diptera (mosquitos) (Herniou et al. 2012), although Federici (1997) suggested that many more virus can be identified from Lepidoptera. The diseases associated with this type of viral structure are named polyhedrosis and their transmission can occur via occlusion bodies (OBs) present in foods – usually sprayed on the leaves like a biopesticide, or horizontal transmission, when present on the surface of eggs, and vertical transmission, inside the ovum of infected adults (females or males). The baculovirus group is a viable alternative for pest control of agricultural importance and is an important tool within the context of IPM. Many biological control programs use baculovirus as the main biological pesticide. Baculoviruses are host-specific restricted to arthropods. So far, no cases of pathogenicity of a baculovirus to a vertebrate have been reported. The objective of this chapter is to demonstrate the diversity of the Baculoviridae family, to which one of the most important virus used in biological control belongs. We indicate the advantages and disadvantages of using the baculovirus for the biological control of agricultural pests and the safety towards mammals, vertebrates, and other animals of using biological products based on baculovirus. We also list biological control programs worldwide that use baculovirus as a biological pesticide and considerations on the use and application of baculovirus products in the field.

12.2 Diversity of the Family Baculoviridae

A great diversity of invertebrate viruses has been documented. However, baculoviruses have been highlighted as one of the most important entomopathogenic viruses with great potential to be used in IPM.

According to the Organisation for Economic Co-operation and Development (2002) and Ikeda et al. (2015), baculoviruses are arthropod-specific viruses and are the only ones lacking homology with viruses found in other organisms such as plants, animals, fungi, and bacteria. Baculoviruses belong to the family Baculoviridae and are one of the largest groups of viruses showing high complexity of forms and functions, besides diversity related to their size, organization, and the gene content of their genomes. The high genomic variation may reflect the phenotypic diversity observed among the four genera of the Baculoviridae family (Herniou et al. 2003; Jehle et al. 2006; Van Oers and Vlak 2007; Miele et al. 2011). In addition to the morphological differences of the granulovirus (GV) and nucleopolyhedrovirus (NPVs), OBs are found in lepidopteran-specific baculoviruses. Figure 12.1 shows NPV and GVs identified from fall armyworm (Valicente et al. 1989).

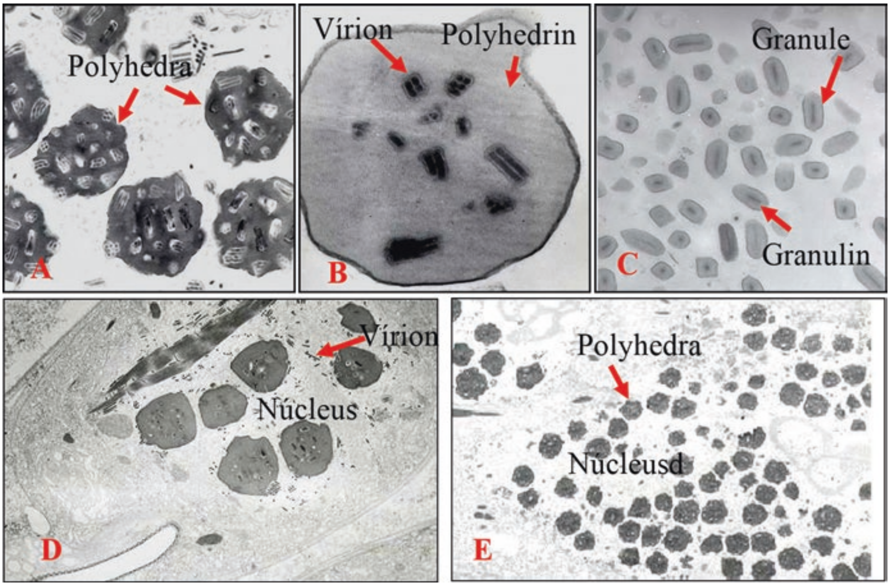

Fig. 12.1 Electron microscope photographs of infected tissues of fall armyworm, *Spodoptera frugiperda*, with baculovirus. (**a**, **b**) Infection of fall armyworm tissue with nucleopolyhedrovirus (NPV) where polyhedra, virion, and polyhedrin can be observed. (**c**) Granulovirus (GV), where only one capsid per envelope and the granulin it is observed; (**d**, **e**) infected tissues with nucleopolyhedrovirus, virions, and polyhedra in the cell nucleus. (Photos: Dr. E.W. Kitajima)

Alphabaculovirus presents cell infection in virtually all tissues of the host insect (Katsuma et al. 2012). The heterogeneity of phenotypes is commonly maintained in field populations, and it is believed that genetic diversity has advantages for the adaptation, evolution, and survival time of baculovirus in the field.

According to Miller and Ball (1998) and Harrison and Hoover (2012), the family Baculoviridae has double-stranded circular DNA with genomes ranging from 80 to 180 kbp, which are packaged in rod-shaped infective particles or nucleocapsids. These are found within proteinaceous bodies called occlusion bodies. For the NPV, polyhedrin is the main constituent of their crystalline protein structure, presenting polyhedral forms called polyhedrins, or granulin with ovoid shape (granules) for the granulovirus.

The VIII Report of the International Committee of Taxonomy of Viruses (Fauquet et al. 2005) classified the family Baculoviridae in two genera until 2015: Nucleopolyhedrovirus and Granulovirus. A new taxonomic subdivision was proposed, which has resulted in the current classification of the Baculoviridae family into four genera: Alpha-, Beta-, Gamma-, and Deltabaculovirus. Alphabaculovirus includes all lepidopteran NPVs that form the viral phenotypes budded virus (BV) and occlusion-derived virus (ODV), Betabaculovirus comprises lepidopteran GVs that also form viral particles during infection, Gammabaculovirus are

Hymenoptera-specific and encompasses NPVs that do not have genes corresponding to the specific proteins of the BV particle, and Deltabaculovirus includes dipteran baculoviruses that do not have in its genome a gene homologous to that encoding polyhedrin expression, characteristic of the other NPVs. According to Eberle et al. (2012a, b), most species of baculovirus are found within the Alpha- and Betabaculoviruses. The largest number of the over 600 known species is from the Lepidoptera.

Figure 12.1 shows the basic structure of a nucleopolyhedrovirus (NPV) and a granulovirus (GV) in an electron microscope of fall armyworm, *Spodoptera frugiperda*, infected tissues.

The life cycle of a baculovirus is pH dependent and the caterpillar ingests the contaminated food with polyhedral inclusion bodies (PIBs). The alkaline pH of the midgut triggers the polyhedra to dissolve and release of one or more nucleocapsids into the midgut lumen (Adams and McClintock 1991; Hasse et al. 2015). According to Hasse et al. (2015) the life cycle starts when a baculovirus occlusion body (OB) is ingested with contaminated food, and when these OBs reach the alkaline midgut, the proteinaceous matrix is dissolved releasing occlusion-derived virus (ODV). The peritrophic membrane is degraded by virus- and host-encoded enzymes present in the OB allowing the ODV to enter the cell, and the ODV enters the cell by fusion with epithelial cell microvilli releasing nucleocapsids (NCs) into the cytoplasm. Nucleocapsids may enter the nucleus, disassemble, and release the genome. At this point, early genes are transcribed and translated, and some of the proteins translocate into the nucleus and take part in genome transcription/replication, NC, and virion assembly. In the first stages of viral infection, NC is transported to the cytoplasm, approaches the basolateral cell membrane (CM), and emerges as budded virus (BV) in the spots where the viral envelope fusion protein (EFP) accumulates, using the secretory pathway. In the very late stages of infection, NCs are enveloped in the nucleus and occluded in the polyhedral-shaped protein matrix (OB) (Hasse et al. 2015).

12.3 Advantages and Disadvantages of Using Baculovirus for the Biological Control of Pests

The great advantage of baculoviruses, which are natural control agents, is that they do not cause any harm to the health of applicators; do not kill natural enemies of insect pests; do not destabilize the environment; do not pollute forests, springs; and rivers; and do not contaminate products *in natura* to be sold in the shelves of supermarkets, leaving no residues in flowers, fruits, and vegetables. All these factors combined with the specificity and ease of handling of baculovirus in relation to chemical pesticides and the target insect make it one of the best biological control agents. The specificity of baculoviruses is a great advantage; however, it can be considered a disadvantage, sometimes infecting only one insect species at a time.

Another advantage of baculovirus-based biopesticide is that they can be sprayed using the same equipment for application of chemicals, but respecting the final volume per hectare. This is a factor that contributes to low-cost application of the baculovirus that does not require special equipment. Several baculovirus-based products have already been tested with chemicals with some good results (Valicente and Costa 1995). Most chemicals have compatibility to be mixed up with baculoviruses, so the use of the same equipment is not a limiting factor, which facilitates the handling within the rural properties.

The action of the baculoviruses is slower than chemical insecticides, taking more time to kill the target insect. Although baculovirus-contaminated larvae reduced their feed by 93% (Valicente 1989), this insect still feeds on the sprayed plant for a couple of days, until it drastically reduces feeding. This factor may contribute to the fact that some farmers do not see the immediate effect of baculovirus in controlling caterpillars, sometimes delaying the incorporation of this control agent into the IPM. In general, baculoviruses infect caterpillars, e.g., fall armyworm, and cause their death as long as they are up to 1 cm in length or at most in the third instar. Thus, soon after planting, crop areas should be monitored to prevent caterpillars from growing and lodging in the corn whorl. In this case, it is very difficult for a biological product and even chemical to reach the caterpillar inside the whorl.

The tendency of a disease caused by baculovirus to become an epizootic depends on the scale of dispersion of this pathogen, the persistence of the virus inside and outside the host, which requires several generations of the host to develop and spread the disease, and it also depends on the number of the target insects present in the field and its stage of development. The instar of the insect is directly related with the rate of infection in the field. Dead larvae represent a relevant source of inoculum for the occurrence and maintenance of epizootics in epidemic populations. Other insects and birds can feed on these dead insects, promoting the dispersion of viral particles into the environment, as well as the rain and wind. The OBs, which present themselves in an environmentally stable way, persist dormant and viable in soil, surviving for many years (Jaques 1967; Thompson et al. 1981). Thus, insect migration and host population fluctuations are events that strongly influence the persistence of the virus in the field, hence assuming an important role in the ecology of baculoviruses.

The population dynamics of insects, considering more broadly the mechanisms of interaction between pathogen and host or the impacts of these pathogens on insect populations in the field, have been little explored. However, the action and influences of parasitoids, predators, and pathogens on maintaining pest density below the threshold of economic damage or reducing their outbreaks have been known and documented.

In most cases, baculoviruses are very efficient because they are characterized as highly virulent and specific to their hosts, as well as being safe for human health and the environment, since they do not cause negative impacts to plants, mammals, birds, fish, or even nontarget insects. In the past 20 years, safety tests have been done and no health or environmental problems have been documented (Hauschild et al. 2011). Baculoviruses have been included in lists of low-risk biocontrol agents, as described in the Organisation for Economic Co-operation and Development (OECD) document entitled Consensus Documentation Information Used in the Assessment of Environmental Applications Involving Baculovirus (http://www.rebecanet.de).

12.4 Safety of the Use of Biological Products Based on Baculovirus

Burges et al. (1980) and the Organisation for Economic Co-operation and Development (2002) published about the safety of baculoviruses stating that NPVs are harmless or are unable to replicate in microorganisms, noninsect cell cultures of invertebrates, vertebrate cell culture, non-arthropod plants and invertebrates. At the end of the 1960s, a NPV isolate for the control of *Helicoverpa zea* (Boddie) (Noctuidae) was subjected to a series of tests as stringent as the chemicals that are submitted by the World Health Organization (WHO) and the U.S. Environmental Protection Agency (USEPA). Tests were performed on primates and on human for carcinogenic and teratogenic effects. The tests were extremely rigorous in order to be sure of noninfection of vertebrate and human animals with insect viruses (Burges et al. 1980). Organisms tested include sparrows, rats, and nontarget organisms (predatory insects, etc.). No adverse effects were detected in any of the organisms tested. Summers and Kawanishi (1978) and the Organisation for Economic Co-operation Development in 2002 published extensive studies confirming biosafety in the use and application of baculovirus-based products in agriculture.

12.5 Biological Control with Baculovirus

Worldwide, there are several biological control programs. There are many examples of Betabaculoviruses, Alphabaculovirus, and Gammabaculovirus that have been developed as microbial control agents and used in a wide range of crops such as corn, potato, citrus, cotton, pasture grass, and tomato (Grzywacz 2017).

12.6 Some Examples of Biological Control Programs Using Baculovirus

It is worth mentioning that all the production process of a baculovirus-based biopesticide is done in the laboratory using healthy larvae from artificial rearing. It is not always easy to complete the life cycle of the caterpillar in the laboratory, having an artificial diet as a limiting factor, and the incubation temperature of the insect after infection with baculovirus is a challenge. Herein, we describe some new biological control programs.

12.7 *Anticarsia gemmatalis* Nucleopolyhedrovirus (AgMNPV) in Soybean Crops

According to Sosa-Goméz (2017), Brazil treats the largest area of soybean with microbial agents, and it includes the baculovirus. This was the largest biological control program using entomopathogenic viruses in Brazil (Moscardi 1999; Moscardi et al. 2011; Sosa-Gómez et al. 2014; Sosa-Goméz 2017). The soybean caterpillar, *Anticarsia gemmatalis* Hübner (Noctuidae), is one of the main pests of soybean cultivation in Brazil, occurring from Argentina to southeastern of the United States. The application of the product reached almost two million hectares of soybeans in the country (Moscardi 1999, 2007; Moscardi and Sosa-Gomez 2007; Moscardi et al. 2011) and counted with the help of researchers from Embrapa and extensionists. However, due to some technical problems, the production by the company Coodetec was closed (Dr. Flávio Moscardi – personal information). Nowadays, *Baculovirus anticarsia* is produced for an area of approximately 300,000 ha/year (Dr. Bráulio Santos – personal information). This virus (AgMNPV) is considered the prototype of baculovirus in relation to biological control because of its importance in both basic and applied research. Extensive studies have been carried out regarding the morphological identification, genetic and molecular characterization, pathology, and biological activity of different AgMNPV isolates. The complete genome of the purified clone AgMNPV-2D has been published since 2006 (Oliveira et al. 2006).

12.8 *Spodoptera frugiperda* Nucleopolyhedrovirus (SfMNPV) in Corn Crops

A very important project started in the 1980s and has developed some baculovirus-based biopesticides. The work with the baculovirus to control fall armyworm began in 1984 at Embrapa Maize and Sorghum in Brazil. A survey of the natural enemies of this pest was carried out in several maize-producing regions of the state of Minas Gerais. During the survey, between 1984 and 1989, more than 14,000 caterpillars were collected, where several parasitoids of the Diptera and Hymenoptera orders were found, including several dead larvae killed by baculoviruses (Valicente 1989). Currently, the baculovirus collection for fall armyworm has 22 isolates sampled in several regions of Brazil. These isolates were studied and characterized and their efficiency evaluated in relation to the cartridge caterpillar (Barreto et al. 2005).

Among the most studied and efficient isolates in the control of this pest, isolate 19 had its genome already fully sequenced (Wolff et al. 2008). The baculovirus that infects fall armyworm larvae causes the disruption of the integument of the insect immediately after their death due to the action of two genes: cathepsin (v-cath) and chitinase (chiA) (Hawtin et al. 1997). Isolate 6 from the Baculovirus Collection of Embrapa Maize and Sorghum presents a unique characteristic of not causing liquefaction of the integument immediately after larva death. Sequencing of the chitinase A (v-chiA) gene from this isolate (SfMNPV-6) showed a mutation in the gene that generates a premature stop codon, considerably reducing the size of the putative enzyme (Valicente et al. 2007a, b, 2008; Vieira 2012). When the insect tegument ruptures, dead larvae infected with baculovirus need to be frozen so that they are harvested with forceps and frozen again until processing and/or formulation. This factor implies greater expenditure of labor, electricity, freezers, and physical space, which results in a final product with a higher price. The advantages of an isolate that does not cause the disruption of the integument is that they are easy to harvest and there is no loss of PIBs.

Many bioassays were performed to characterize this baculovirus isolate such as lethal concentration 50 (LC_{50}), lethal time 50 (TL_{50}), and several other factors such as polyhedra produced per caterpillar, larval equivalent (which is the number of larvae needed to spray 1 ha – LE/ha), and weight larval equivalent (which can be defined as the weight of larvae required to be sprayed in 1 ha). Thus, the main objective is to produce more polyhedra per larvae and, consequently, to reduce the number of equivalent larvae (LE) (Valicente et al. 2013). Valicente et al. (2013) also found a strong correlation between weight and the number of dead larvae that needed to be sprayed in 1 ha. Thus, between 12 and 14 g of dead larvae equivalent is needed to be sprayed in 1 ha. Nowadays, there are contracts with 3 large companies for this product to be on the market. The first Brazilian company to register the *Baculovirus spodoptera* for fall armyworm was VR Biotech®, and the name of the commercial product is Cartuchovit®. This isolate was also registered by Simbiose®, also a Brazilian company, named VirControl® (Fig. 12.2).

12.9 Baculovirus for the Control of the Soybean Looper, *Chrysodeixis* sp.

Embrapa Maize and Sorghum in Brazil has a collection of more than 50 baculovirus isolates that cause typical symptoms (Fig. 12.3). Each isolate has already been tested in the laboratory and there are projects with partner companies in which there is a development for large-scale production of commercial products (Simbiose® company). The characterization of the best isolate has been done and this biopesticide should be on the market in 2018/2019. Another research unit of Embrapa (CENARGEN), since 2008, has also been developing studies of biological and molecular characterization and pathogenicity evaluation (in collaboration with Embrapa Soybean).

Fig. 12.2 Baculovirus-based biopesticides registered in Brazil for fall armyworm, *Spodoptera frugiperda*. Both formulations are wettable powder. (Photo: Fernando H. Valicente)

Fig. 12.3 Dead larvae of soybean looper, *Chrysodeixis* sp., with a typical symptom caused by baculovirus. (Photo: Priscila Marques de Paiva)

12.10 Nucleopolyhedrovirus for the Control of *Helicoverpa armigera*

Helicoverpa armigera larvae were collected in some regions of Brazil as soon as the outbreak of this pest occurred between 2012 and 2013. Larvae were brought to the laboratory and several baculovirus isolates were discovered. These isolates were tested on healthy caterpillars, obtaining the same initial symptoms, and some isolates were identified through primers and were sequenced. The comparative analysis of the sequencing for the genes LEF-8 and LEF-9 showed that the isolates found in Brazil are closely related to the isolates from Australia and India. All baculovirus isolates tested caused a good mortality rate in larvae of *H. armigera* third instar. However, lethal concentration 50 and lethal time 50 varied among these isolates. All of our isolates showed to be HearNPV and not HzNPV (Gemstar) according to the DNA sequencing. HearNPV-BR2 showed the best results for LC_{50} and LT_{50}. Thus, this was the first report of baculovirus isolates infecting *H. armigera* larvae in Brazil, and also the first report of *H. armigera* baculovirus isolates to be identified as HearNPV in Brazil. Isolate BR2 showed the best results and will be used in *H. armigera* biological control programs due to its characteristics. An agreement with Simbiose® company was signed for large-scale production of this biological pesticide. Figure 12.4 shows a caterpillar killed by the baculovirus, with typical symptoms.

Fig. 12.4 Dead larva of *Helicoverpa armigera* with typical symptoms of baculovirus. (Photo: Arthur Torres)

12.11 Considerations in the Use and Application of Field Baculovirus Products

Some considerations should be made for the best use and storage of baculovirus in the form of biopesticide. The baculovirus-based bioinsecticide should be stored in a cool dry place without direct sunlight for better preservation, maintaining the product quality. Wettable powder formulations do not need to be stored in the freezer. Timing of the application of the baculovirus should be done according to the pest and with the respective crop. For example, the timing of application of *H. armigera* baculovirus is different in maize, soybean, and cotton. *H. armigera* attacks at different times, different parts of the plant, and different stages in relation to the development of each crop. In the specific case of the fall armyworm, observe the first injured leaves or the location presence of this pest through the years. In very warm regions with a history of pest attack, the first application of the baculovirus against the fall armyworm should be made always between 10 and 15 days after germination. It is important to monitor the presence of the insect pest, since the timing of the application of the baculovirus for the first application is very important; the smaller the caterpillar, the greater the chances of control. Overlapping of larval stages should be avoided. Spraying should be performed after 4 pm due to the lower incidence of ultraviolet (UV) rays. In large areas where there is a need for continuous spraying during the day, it is best to use a baculovirus formulation, if available, with protection against UV rays. Viral particles exposed to sunlight or high temperatures can be inactivated quickly. The amount of the water to be sprayed in the field should be adequate according to the spraying technology and for each crop. However, it must be ensured that there has been adequate deposition of the product on the leaf of the crop in question, specifically for maize, especially in the region of the whorl. It is not efficient to use a very low volume of water to spray in order to have a high field efficacy, and the product is not deposited on the leaves. In the case of organic products, the biological pesticide to be sprayed and its mode of action must be respected. The insect must ingest part of the sprayed leaf to become contaminated, so an inadequate spraying technology cannot be used. To use a smaller amount of water, one should have the appropriate equipment for low volume, but check the relative humidity of the air because if it is very low, you can lose much with the evaporation of the water before the product reaches the leaves (Fig. 12.5). All applications of the baculovirus-based biopesticide should be used with an adhesive spreader that is compatible with the biological product as it improves the distribution and adhesion of the product on the leaves.

The application of baculovirus in corn, soybean, and cotton crops should follow some important factors. The first one is that the crop should be monitored weekly to detect the level of attack of the target pest in the crop. The attack of larvae begins at different times of the growing stage of each crop. Therefore, the timing of the baculovirus application is important for each pest and is specific for each crop.

Another important factor is the architecture of the plant and the leaves to be sprayed by the baculovirus or any other biological product. In the case of maize that grows vertically, if necessary, weekly applications of the biopesticide should be carried

Fig. 12.5 Application of baculovirus with an appropriate equipment and volume of water. (Photo: Fernando H. Valicente)

out in the same way, as needed, as it is done with the use of chemicals. Because every week there will be new leaves without any biological product coverage.

12.12 Final Remarks

Baculoviruses are arthropod-specific viruses and are the only ones lacking homology with viruses found in other organisms such as plants, animals, fungi, and bacteria. Although the action of the baculoviruses is slower than chemical insecticides, taking more time to kill the target insect, it is considered an important and powerful tool in the integrated pest management. Some important aspects should be considered for the use and field application of baculovirus products, as well as storage. There are many biological control programs in the world that use baculovirus as biopesticide with positive results. These programs have been expanding as the insect pests are moving throughout the world such as fall armyworm that is present in African countries and now in India. Fall armyworm is present in Latin American countries.

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