20

Baculoviruses: Members of Integrated Pest Management Strategies

Vanina Andréa Rodriguez,
Mariano Nicolás Belaich and Pablo Daniel Ghiringhelli
LIGBCM-AVI (Laboratorio de Ingeniería Genética y Biología Celular y Molecular Area Virosis de Insectos)
Universidad Nacional de Quilmes/Departamento de Ciencia y Tecnología
Argentina

1. Introduction

Integrated Pest Management (IPM) can be defined as "an ecologically based pest control strategy that relies heavily on natural mortality factors and seeks out control tactics that disrupt these factors as little as possible" (Flint & Bosch, 1981). IPM began to be applied because the extensive use of chemical insecticides show different types of environmental damages, as development of resistant insects, the appearance of new pests, injury to bird and mammals populations and human health damage due to the release of toxic waste on environment and food contamination. The aims of IPM are to protect crops, with minimum cost and risk for humans, animals and ecosystems. The development and application of IPM requires the knowledge of how the ecosystem influences on pest insects and its natural control agents, and how to modify this environment to control particularly pest insects and avoid the related chemical agent's problems.

2. Integrated pest management (general overview)

IPM applies different tactics, like pest resistant plants, use of entomopathogens such as bacteria and viruses, and strategies that involves cultural, physical, mechanical, biological and chemical control. The use of these combined tactics reduces the chances of generating resistance and insect survival.

2.1 Pest resistant plants (transgenesis using plant genes)

Plants have a vast metabolic capability and produce many secondary chemicals which are toxic, anti-nutritional, or aversive to species might otherwise be potential predators (Norris & Kogan, 1980). Examples include the pyrethrins from chrysanthemums and alkaloids like nicotine from tobacco. Other compounds implicated in protection from insect attack include the terpenoids, steroids, flavonoids, phenolic, glucosinolates, cyanogenic glycosides, rotenoids, saponins and non protein amino acids (Gatehouse *et al*., 1991). As secondary compounds are the products of multi-enzyme pathways which involve the interaction of many gene products, such defense system are in most cases too complex to be used in plant

genetic engineering (Dawson *et al*., 1989; Hallahan *et al*., 1992). However, a few plant defense mechanisms are based on the product of a single gene, and the target site of many is the insect digestive system. Most of these types of single genes are suitable for gene transfer. Being of plant origin, they have the advantage that they are likely to have a high degree of compatibility with the metabolic system of the transgenic host plant.

Crops resistant to insect attack offer an alternative strategy of pest control upon chemical pesticides. Transgenic plant technology can be a useful tool in producing resistant crops, by introducing novel resistance genes into a plant species. Several different classes of plant proteins have been shown to be insecticidal towards a range of economically important insect pests. Genes encoding insecticidal proteins have been isolated from various plant species and transferred to crops by genetic engineering. Amongst these genes are those that encode:

1. Protease inhibitors (serine and cysteine). The damage of leaves of certain solanaceous plants, either by insect feeding or mechanical wounding, induced the synthesis of protease inhibitors (Green & Ryan, 1972; Shumway *et al*., 1976; Walker-Simmons & Ryan, 1977; Brown *et al*., 1985). The first gene of a plant successfully used to be transferred to another plant was a trypsin inhibitor (cowpea trypsin inhibitor, CpTI) (Pusztai *et al*., 1992; Graham *et al*., 1995; Xu *et al*., 1996). As an example of a commercial deployment of a proteinase inhibitor transgene to date, could be mentioned the culture of genetically engineered cotton varieties in China. These varieties express two transgenes to improve cotton protection, Bt toxins against lepidopteran larvae and CpTI. In 2005, Bt/CpTI cotton was grown on over 0.5 million hectares (Gatehouse, 2011).
2. Inhibitors of α-amylase. This enzyme is able to catalyse the hydrolysis of α-1-4 glycosidic bonds, transforming polysaccharides into mono and disaccharides (Grossi-de-Sá & Chrispells, 1997; Franco *et al*., 2002; Pelegrini *et al*., 2006). Interference of the carbohydrate absorption could be a way to reduce the insect pest feeding (Yamada *et al*., 2001). Leguminous seeds are known as rich sources of proteinaceous α-amylase inhibitors (α-AIs) (Payan, 2004). Expression of α-amylase inhibitors (*a*-AIs) from both scarlet runner bean (*Phaseolus coccineus*) and common bean (*Phaseolus vulgaris*) has been shown to be effective in transgenic plants, showing high protection against seed weevils in pea (Shade *et al*., 1994; Schroeder *et al*., 1995), azuki bean (Sarmah *et al*., 2004), chickpea (Sarmah *et al*., 2004; Ignacimuthu & Prakash., 2006), cowpea (Solleti *et al*., 2008) and coffee (Barbosa *et al*., 2010).
3. Lectins. This group of carbohydrate-binding proteins constitutes entomotoxic factors present in many plant species. During the last decade a lot of progress was made in the study of the properties of a few lectins that are expressed in response to phytophagous insects. Based on their activity towards pest insects, these proteins have a high potential for use in pest control strategies. For example, the use of plant lectins has been applied to control numerous pests: melon fruit fly larvae (Kaur *et al*., 2009); *Aedes aegypti* larvae, which has developed tolerance to many other insecticides (Coelho *et al*., 2009); and the cotton leafworm *Spodoptera littoralis,* an economically important caterpillar in agriculture and horticulture (Hamshou *et al*., 2010).

2.2 Entomopathogen bacteria (*Bacillus thuringiensis*)

Bacillus thuringiensis is a Gram positive bacteria belonging to Eubacteria. It was isolated in the early twentieth century in Japan from dead larvae of the silkworm. This bacteria, produces spores and crystalline bodies composed of one or more proteins with insecticidal activity

(Schnepf *et al*., 1998, Sedlak *et al*., 2000). The crystalline toxins, named Cry δ-endotoxins, exist in a variety of forms: bipyramidal, spherical, rhomboidal, cuboidal and irregular, among others, and are active against a large number of insect groups as well as nematodes and protozoa. Today there are over 40 groups of Cry proteins (Crickmore *et al*., 2009). The δ-endotoxins are synthesized as an inactive pro-Cry-toxin and when they are ingested by the larvae feeding on plant debris or soil, the inclusions are solubilized in the alkaline conditions of the digestive tract of the larvae and are converted by the action of the insect proteases in active peptides (Feitelson *et al*., 1992; Schnepf *et al*., 1998). Active toxin is recognized by a specific receptor; it binds to microvilli of intestinal cells (Gazit *et al*., 1998; Gerber & Shai, 2000), and generates ion channels. The natural ion imbalance dissipates and the pH of medium diminishes causing osmotic cell lysis and larva ceases to feed (Schnepf *et al*., 1998). Moreover, tissue destruction allows mixing the contents of gastrointestinal tract with hemolymph. Both phenomena favor the germination of bacterial spores, resulting in the death of the larva few days after ingestion crystals (Aranda *et al*., 1996; Schnepf *et al*. 1998; Crickmore *et al*., 2009).

There are two main approaches to use Bt as a pest control agent:

1. Preparations based on living or dead Bt containing spores and crystals are sprayed on crops, as if it were a conventional insecticide. This strategy are currently used in the United States, Europe, Argentina and Mexico as a biological control for insect and other invertebrate pests (mites, nematodes, flatworms and protozoa) that affect crops of corn, potato, tomato, sorghum, rice, coffee, beans, sugar cane, among others (Neppl, 2000). The application of Bt in insect control is not exempt from the emergence of resistance. Using of a combination of toxins reduce resistance to individual toxins, maintains a populational balance and prevents the prevalence of resistant variants (Georghiou & Wirth, 1997; Ives *et al*., 2011; Yang *et al*., 2011). Research and years of use have shown that the employment of Bt products is not hazardous to non-target arthropods, birds, fish, mammals, or environment (EPA, 2008).
2. The genes that encode different Cry proteins have been used to generate genetically modified (GM) plants. Transgenic plants are resistant to the attack by insect pests. The most widely GM crops commercialized so far include mainly maize, cotton and rice. The use of this modified crops has been approved in several countries, including United States, Brazil, Argentina, Canada, Australia, Spain, South Africa, among other (James, 2011). Considering that insecticidal crystal proteins can be released continuously into the soil in different forms during the growing period of Bt-plants (Zhou *et al*., 2011), biosafety of the use of genetically modified plants is always questioned. However, data regarding the development and commercial use of transgenic Bt varieties have shown that the currently available Bt crops have no direct detrimental effects on non target organism due to their narrow spectrum of activity. In addition, the use of these modified crops, such as Bt maize and Bt cotton, results in significant reductions of insecticide application and has clear benefits on the environment and farmer health. Consequently, Bt crops can be a useful component of IPM systems to protect the crops from targeted pests (Yu *et al*., 2011).

2.3 Entomopathogen viruses (*Baculoviruses*)

The insect viruses are intracellular parasites that can only reproduce inside a susceptible insect host. They are valuable natural control agents, providing a secure control, effective

and sustainable in a variety of insect pests. The virus particles are present in the environment and usually can be found on the surface of plants or in the soil. Insects become infected by consuming plant material contaminated with viral particles on the surface or by contact with the soil. Baculoviruses are the most common type of insect viruses. It has been reported that infect over 600 species of insects worldwide. Most baculovirus infect caterpillars, the immature form of moths and butterflies. Naturally, these viruses are potent regulators of the population of many caterpillar pests, but the use as a tool for biological control in agriculture is limited by biological or technical reasons.

1. Advantages. Insect viruses are very safe to handle, since they are not infectious to organisms other than their natural hosts. Moreover, most insect viruses have a high specificity, so that the risk of affecting non-target beneficial insects is very low.
2. Disadvantages. Most insect viruses have a low speed of action on their insect host, during this time the plague is still eating and damaging. Insect death is also dose dependent, and very high doses are often necessary for adequate control. Usually, viruses are very effective against early larval stages; the late larval stages are less susceptible to virus infection. Virus particles exposed to sunlight or high temperatures are rapidly inactivated. In addition, some cultural practices can affect viral persistence, hiding the viral particles in the soil.

3. Baculovirus

3.1 Baculovirus biology

Baculoviridae is a viral family that infects insects. Their genomic material is composed by double strand, circular, super coiled DNA, with a size ranging from 80 to 180 kpb. These viruses are classified in four genera: *Alphabaculovirus* (nucleopolyhedroviruses –NPVs- that specifically infect insects of lepidopteran order), *Betabaculovirus* (granuloviruses -GVs- that specifically infect insects of lepidopteran order), *Gammabaculovirus* (NPVs that specifically infect insects of hymenoptera order) and *Deltabaculovirus* (NPVs that specifically infect insects of diptera order) (Jehle *et al*. 2006). The virus genome is packaged within a rod-shaped nucleocapsid which is further surrounded by a lipoprotein envelope to form the virus particle. This structure is then occluded at very late stages by a crystalline matrix (Occlusion Body or OB) largely comprising for a single occlusion protein (about 28 kDa), which serves to protect it in the environment. During the infection cycle two different phenotypes are generated: budded virus (BVs, responsible for the systemic cell to cell infection) and occluded-derived virus (ODVs, responsible for the host to host infection) (Figure 1).

The most common way of insect host primary infection is by ingestion during larval feeding of contaminated foliage (Figure 2). Following virus ingestion, the OBs are dissolved in the high pH conditions (pH 8.5 to 11) of the insect midgut, releasing the virus particles (ODVs) into the gut lumen (Granados & Lawler, 1981; Pritchett *et al*., 1981; Pritchett *et al*., 1984; Rohrmann, 2008). Released viruses bind to the columnar epithelial cells and enter the tips of the microvilli on the apical brush border of cells (Kuzio *et al*., 1989; Faulkner *et al*., 1997; Haas-Stapleton *et al*., 2004). Following fusion between cell and virus membranes, the nucleocapsids are released into the cytoplasm and are transported to the nucleus, where

viral DNA transcription and replication occurs. Into the cytoplasm, nucleocapsids are transported from the basal membrane to the haemocoel, acquiring the host derived membrane and virus encoded proteins (Washburn *et al*., 2003). Secondary infection is achieved by BVs produced from the midgut cells. Thus, the viruses spread to other insect tissues including the fat body, endodermis, muscle sarcolemma and nerve ganglia (Harrap, 1970; Washburn *et al*., 2003). Prior to death larvae become creamy in colour, cease feeding and show limited movement. In most baculovirus infections, the host tissues break down as a result of the expression of virus-encoded chitinase and cathepsin proteins (Ohkawa *et al*., 1994; Hawtin *et al*., 1995; Slack *et al*., 1995). Then, OBs are released into the environment following the rupture of the insect cuticle; 10^9 OBs may be released from a single larva, and may remain viable in the environment for several years, until ingestion by another host larva resumes replication cycle (Evans & Harrap, 1982) (Figure 2).

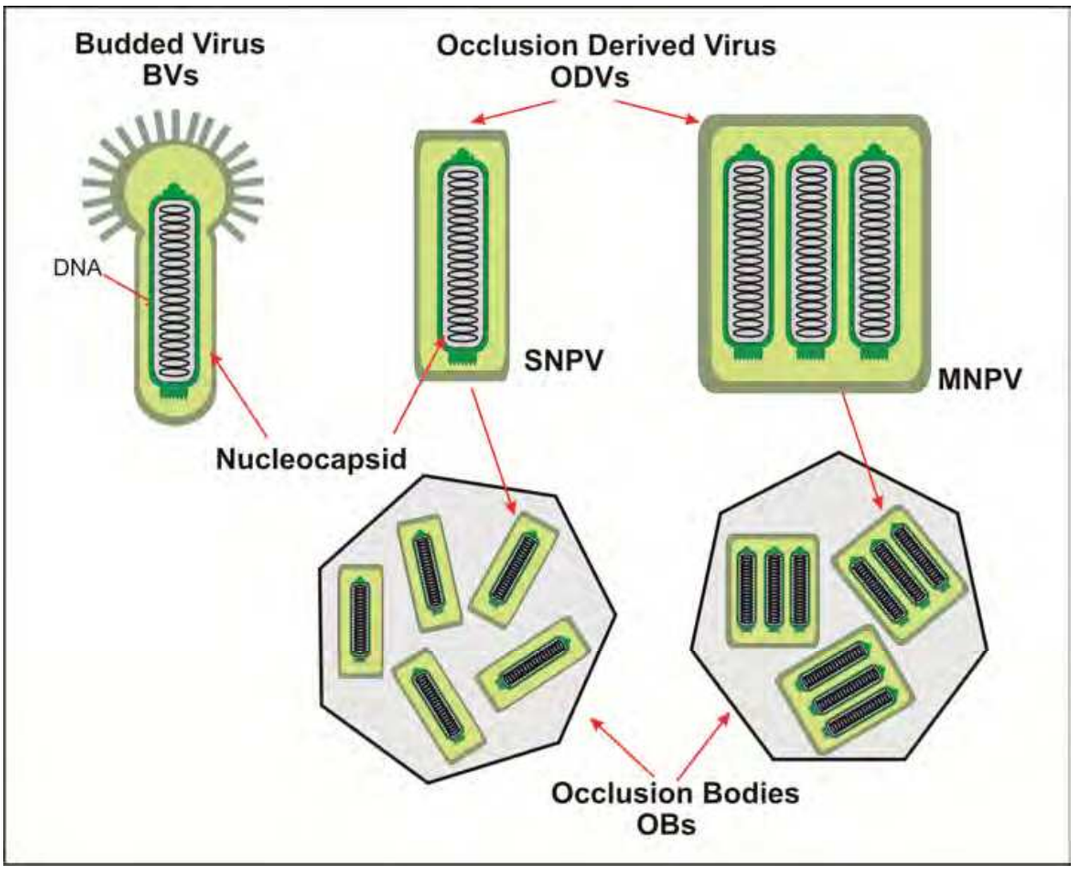


Fig. 1. **Alphabaculovirus phenotypes**. Occlusion bodies (OBs) containing occlusion derived virus (ODVs) responsibles for the primary infection in the midgut cells and Budded virus (BVs) that spread the infection to other larval tissues.

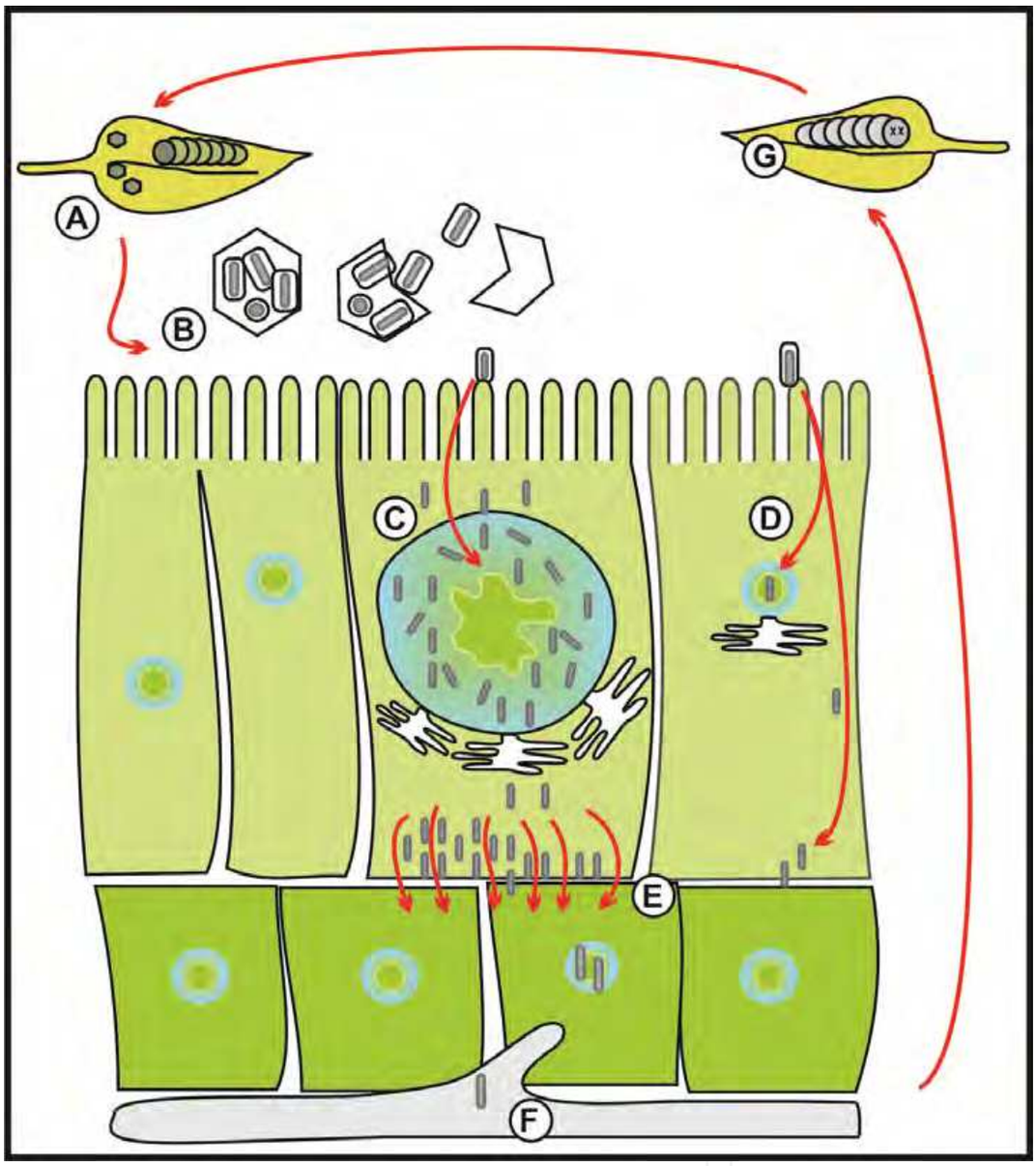


Fig. 2. **Infection cycle.** NPV biological cycle. **A**. Larva ingests a baculovirus-contaminated leaves. **B**. The virions are released and bind to midgut cell microvilli. **C**. Into columnar cells the nucleocapsids are transported to the nucleus, where the viral DNA is released and the BVs are generated. **D**. Nucleocapsids can pass through the cell to the haemocele. **E**. Viral particles enter into susceptible tissue cells and initiate a replication cycle to produce a new BV generation. **F**. Later, the OBs are generated into each infected cell. **G**. Cell lysis occurs, larval integument breaks, and the OBs are released to be eaten by other larva and thus restart the infection.

3.2 Baculovirus as control agents (Figure 3)

Wild type baculoviruses

A large number of Lepidoptera are pests of economically important crops such as soybean, sunflower and cotton, among others. Alpha- and Betabaculoviruses are very useful tools for control of lepidoteran pests. These viruses have a narrow host range, are safe for other insects and organisms in the environment (Szewczyk *et al*, 2006), and have strong pathogenicity and virulence. Other advantages are the stability in the environment for long periods and that can be applied using simple methods. Because of the great advantages of baculoviruses as biological control tools and their safety for the ecosystem, their use in IPM is accepted and is steadily increasing. Currently, different baculovirus are used in pest control management worldwide.

In North America, the use of baculoviruses as a pest control agent started as early as 1930 with the protection of pine trees with *Diprion hercyniae* NPV (Bird & Buek, 1961). Subsequently, this strategy was use to protect numerous commercial crops, including alfalfa, cabbage, corn, cotton, lettuce, soybean, tobacco and tomato (Granados & Federici, 1986). Another baculovirus that is currently used to control pest infection is *Helicoverpa zea* Simple Nucleopolyhedrovirus (HzSNPV). This virus infects several species belonging to the genera *Heliothis* and *Helicoverpa*, which include a wide range of crop pests (Chakrabarti *et al*., 1999). HzSNPV provides a tool for control against the bollworm, soybean, sorghum, corn, tomatoes and beans. In the 90´s of the latest century the virus was registered under the name GemStar™ and marketed by Thermo Trilogy Company. This virus produced in United States is also marketed in Australia by Aventis Crop Science and it is now used to protect crops against *Helicoverpa armigera* (Mettenmeyer, 2002), which attack near 200 crops including cotton, soybeans, chickpeas, sunflower, snuff, pepper, and corn, among others. HaSNPV has been adopted for large-scale viral pesticide production in China and has been used extensively to protect crops of cotton (Zhang *et al*., 1995).

In South America, probably the most successful program of using baculovirus in pest control is the program implemented in Brazil since 1980 to control the velvet bean caterpillar *Anticarsia gemmatalis* (pest of soybean) (Moscardi, 1989, 1999). In this program, the *Anticarsia gemmatalis* Multiple Nucleopolyhedrovirus (AgMNPV) is used as a biological insecticide against *Anticarsia gemmatalis* larvae, and by 2005 the treated area reached 2 million hectares (Szewczyk *et al*., 2006). One advantage of using this virus is that it is highly virulent and only needs to be applied once, whereas chemical insecticides should be applied twice. In addition, the use of this virus is 20 to 30% less expensive than chemical insecticides. Recently, a pilot plant was constructed in Embrapa Soja, Londrina, in order to improve the laboratory process and train people in the production of virus. This laboratory will be able to inoculate 20,000 to 30,000 larvae per day (Szewczyk *et al*., 2006).

Moreover, the *Cydia pomonella* Granulovirus (CpGV) has been used as pest control in crops of apples and pears. Its use has increased in Europe and North America since 2000 and is used in about 100,000 ha in those continents. Currently there are several commercial preparations that include Cyd-X, Virosoft CP4 (North America), Carpovirusine™ (France), Madex™ y Granupon™ (Switzerland), Granusal™ (Germany) and Virin-CyAP (Russia) (Rohrman, 2008). In Argentina, *Cydia pomonella* is a pest of pear, apple and walnut crops. The National Institute of Agricultural Technology (INTA; Argentina) in agreement with the

Natural Plant Protection company (France) developed a bio-insecticide called Carpovirus to control that pest, using a formulation based on CpGV.

For an additional example of baculovirus employed as tools of biological control and pest management, can be mentioned *Spodoptera exigua* Multiple Nucleopolyhedrovirus (SeMNPV). Formulations of this virus are being used to protect crops of sweet peppers in Spain against *Spodoptera exigua*. The company Biocolo SRL is responsible for the commercial production of this bio-insecticide with the capacity to treat 50,000 larvae per day. The introduction of this biological insecticide has helped to multiply more than 15 times the area of biologically protected crops grown, reaching 23,000 ha in 2008 (Caballero *et al*., 2009).

Modified baculoviruses

Despite the advantages that baculovirus have, there are also some limitations for their use as pest control agents. Some of these limitations are the high costs of in vivo production and the low persistence in very sunny conditions (Ignoffo *et al*, 1977). Also, the virus must be used in early insect development stages, because in the late stages the insects are more resistant to infection (Washburn *et al*., 2003). Another limitation is their slow speed to kill the pest (Ignoffo *et al*, 1992). To avoid this, recombinant baculovirus have been developed and offer attractive alternatives to broad-spectrum chemical control. These recombinant viruses can express specific toxins (Inceoglu *et al*, 2001), hormones (Elvira *et al*, 2010) or enzymes (Gramkow *et al*, 2010) and are much more efficient than the wild-type virus in speed to kill.

1. **Expression of insect-selective toxin.** The *aait* gene obtained from *Androctonus australis* scorpion is one of the most promising toxins to be expressed in baculovirus. A recombinant virus containing this gene showed to be 40% faster in killing larvae than the wild type and a reduction of host feeding by 60% (Cory *et al*., 1994; Inceoglu *et al*., 2001). The site of action of this neurotoxic polypeptide is one insect sodium channel. Lepidopterous larvae infected with an AaIT-expressing baculovirus reveal symptoms of paralysis identical to those induced by injection of the native toxin (Elasar *et al*., 2001) and many of the physiological effects are very similar to those of pyrethroid insecticides which also act at the same target (Gordon *et al*., 1992). Other useful insect-selective neurotoxins are SFI1 (obtained from a European spider *Segestria florentina*) and ButaIT (derived from the South Indian red scorpion *Mesobuthus tamulus* (Wudayagiri *et al*., 2001). Some toxins could exert a cooperative effect when they are co-expressed, such as LqhIT1 and LqhIt2, obtained from *Leiurus quinquestriatus* scorpion (Regev *et al*., 2003).
2. **Expression of insect hormone genes.** Disruption, over expression or inactivation of one or more insect hormones results in abnormal growth, feeding cessation and/or death. So, the insertion of genes that encode insect hormones were the first strategies used to generate genetically modified baculovirus. A recombinant virus of *Bombyx mori* MNPV (BmNPV) that encodes an active diuretic hormone (DH) showed to be 20% faster in killing larvae than wild type virus (Maeda *et al*., 1989). Later, by the deletion of *egt* gene, which prevents the larval molt, the mutant virus resulted to be 30% faster in killing larvae and in a considerable reduction in food intake than wild type virus (O´Reilly & Miller, 1991). Also, this gene may be replaced by an exogenous gene and enhance the insecticidal activity (Arif, 1997; Popham *et al*., 1997; Sun *et al*., 2004).

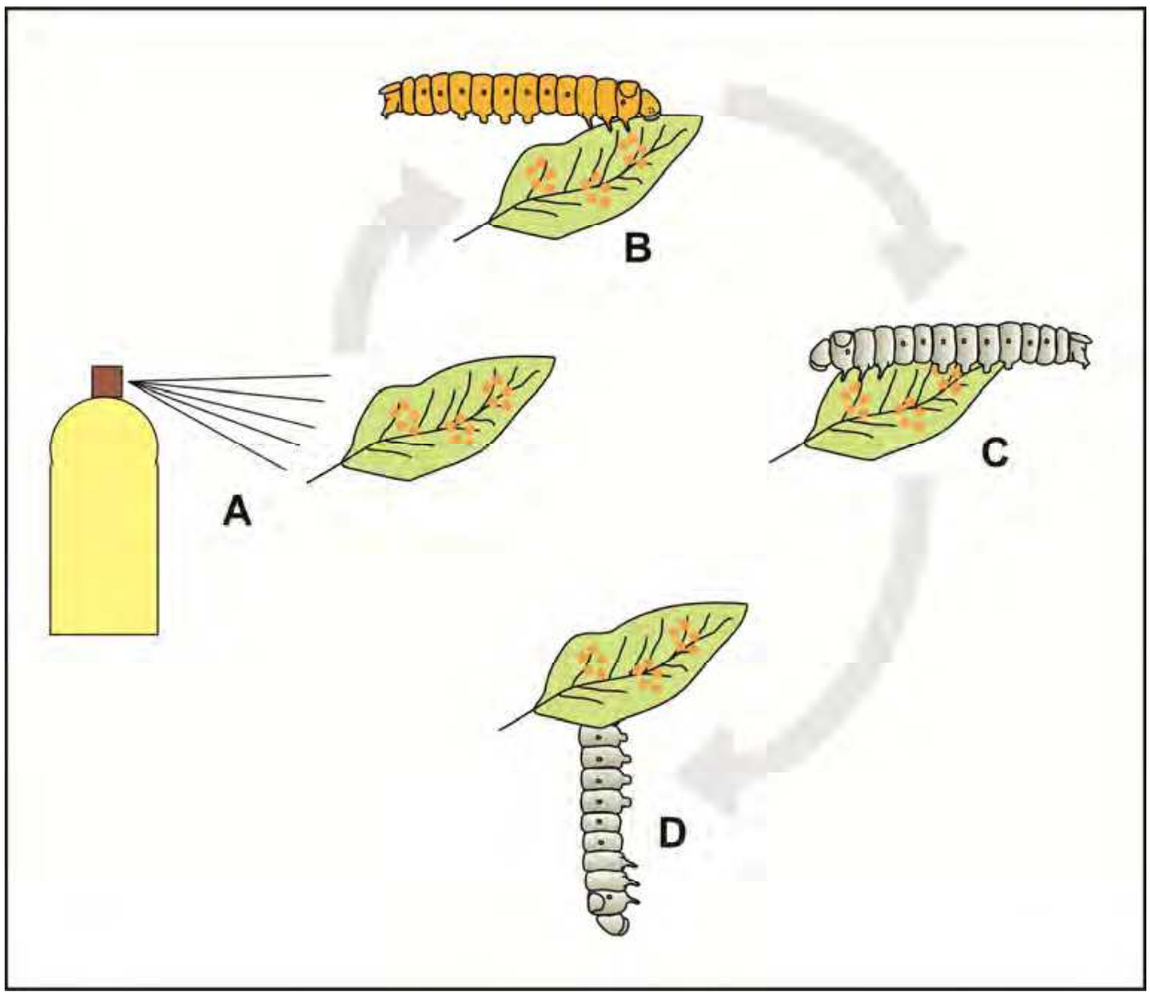


Fig. 3. **Baculoviral bioinsecticide. A**. Bioinsecticide spreading on leaves. **B**. Larvae feeding a contaminated leaf. **C**. Infected larvae. **D**. Dead larvae. Released virus will permit the resume of the infection cyle.

Baculovirus safety

Alpha- and Betabaculoviruses are not infectious for predatory or beneficial insects outside of the order Lepidoptera, or toward other non-targeted organisms (Black *et al*., 1997; Szewczyk et al., 2006). Baculoviruses do not replicate in mammals, birds, fish, amphibians, reptiles and other vertebrates (Barsoum *et al*., 1997). Moreover, different studies have concluded that the transgenes that encode toxins or other foreign proteins in recombinant baculovirus increase the speed to kill the specific target, but do not confer any selective ecological advantage compared with the wild-type baculovirus. (Cory *et al*., 1997; Lee *et al*., 2001). Another concern associated with GM organisms is the possibility of genetic recombination that results in the "jump" of the recombinant baculovirus foreign gene to another organism. If the pesticide based on a recombinant baculovirus is used long enough and in sufficiently high concentrations in the field, it is expected that genetic recombination could occur. In both the field and in laboratory conditions, this phenomenon is expected to

be higher among highly homologous baculoviruses infectious to the same host (Hajos *et al.*, 2000). However, it is important to note that the recombinant virus with a phenotype more virulent than the wild type has disadvantages, since they produces fewer progeny and are rapidily out-competed in the ecosystem (Inceoglu *et al.*, 2001).

Future prospects

In order to improve the use of baculovirus in pest control management, various methodologies are being studied and developed. Among these we can mention:

1. Expression of fusion proteins to expand the host range. Because ButaIT toxin alone exhibits weak oral toxicity, and as an alternative to the use of recombinant baculovirus different strategies have been developed based on the generation of fusion proteins. One of the proteins used is a lectin, which functions as a carrier (GNA, *Galanthus nivalis* agglutinin) and the other protein is a toxin. A fusion protein comprising GNA and SFI1 has been shown to have insecticidal effects on both lepidopteran and homopteran plant pests (Fitches *et al*., 2004; Down *et al*., 2006) while a fusion protein combining GNA with ButaIT has also been shown to have insecticidal activity against lepidopteran larvae. The injection data reported show that fusion proteins containing SFI1 and ButalT are insecticidal towards a range of insects including lepidopteran, dipteran, coleopteran and dictyopteran pests (Fitches *et al*., 2010).
2. Antisense strategies. A new approach to the development of integrated pest control is the use of technologies based on iRNA (Gordon & Waterhouse, 2007; Price & Gatehouse, 2008). For efficient pest control methods using iRNA in the field, the major challenge is the development of easy and reliable methods for production and delivery of dsRNA. The dsRNA can be produced *in vivo* using a bacteria system or can be synthesized *in vitro*. Other way to produce dsRNA is using transgenic plants that express the desired antisense. The delivery of dsRNA can be done by spraying dsRNA on the crop plants or by feeding insects. In a recent study feeding heat-killed bacteria that produced dsRNA or *in vitro* synthesized dsRNA was used to silence five target genes in Colorado potato beetle, *Leptinotarsa decemlineata*. It was observed that the loss of function of these target genes caused larval mortality and significantly decreased insect growth (Zhu *et al*., 2011). In a similar study, it was used a synthetic iRNA to inhibit a mitochondrial electron transport of *Plutella xylostela*, causing prominent insect mortality (Gong *et al*., 2011). The use of different target genes could minimize the risk of resistance development. Offtarget effects and species specificity of dsRNA are two major potential issues that need to be addressed. However, the same way as was observed with *Caenorhabditis elegans* and other plant nematodes (Price & Gatehouse, 2008), in insects, described methods to feed dsRNA to a range of different insect species demonstrated that even highly conserved genes can be exploited to trigger species-specific iRNA without affecting non-target species (Whyard *et al*. 2009).
3. Combined control methods. The use of synergism between two different pathogens is a strategy that could be useful to conduct pest control strategies. To evaluate this possibility it have been conducted studies using *Spodoptera exigua* larvae and assessed the effect of using a baculovirus (*Spodoptera exigua* NPV) and a parasitoid (*Microplitis pallidipe*s) to control this pest (Jiang *et al*., 2011). The results obtained indicate that when *M. pallidipes*-parasitised *S. exigua* larvae were infected by SeMNPV, the virus did not affect the developmental period of *M. pallidipes* in the host, and most parasitoids

completed development, possibly because parasitism by *M. pallidipes* reduced larval sensitivity to the virus. The results of this study also indicate that *M. pallidipes* is an important vector of SeMNPV and contributes to natural epizootics of the virus. Female parasitoids that had developed or oviposited in virus-infected hosts, or that emerged from cocoons contaminated with virus, were able to transmit infective doses of virus to healthy host larvae (Jiang *et al*., 2011).

4. Conclusions

Considering the amount of baculovirus species that have been isolated so far, its development as bio-pesticides has not been commensurate with all its potential. Most of the viruses found in commercial phase are produced by small or medium companies or by the users themselves, as is the case of USDA Forest Service (US Department of Agriculture), CIP (International Potato Center) or EMBRAPA (Empresa Brasilera de Pesquisa Agropecuaria). Among the factors limiting their commercialization it has been noted that they are too specific, they present a slow speed of action, they have a low persistence in the field, and are costly to produce infecting larva. Also, the approaches that involve *in vitro* production processes in insect cell cultures are still in development stage.

An entomopathogenic product will be considered as a viable alternative in pest control if it meets control with the same speed, ease of use, at the same cost than a chemical insecticide. This way, do not take into account their unique capabilities: the ability to replicate in their host and be dispersed in culture, the ability to synergistically act with natural enemies, and availability to be produced locally or regionally. Faced with this situation have been conducted many studies to increase the speed of action, to extend the host range and maintain their safety to non-target organisms. This involves the generation of genetically modified baculoviruses and the use of different viral formulations (Cherry & Williams, 2001).

Baculovirus survival in the environment can be affected by temperature, pH, moisture, the presence of additives, exposure to UV light, and by the action of some plant metabolites such as peroxidases, that generate free radicals (Hoover *et al*., 1998; Zhou *et al*., 2004).

Actually, there have been developed some protective agents against UV that have been included into the viral formulations. Some commercial bleach such as Phorwite AR, Blankophor, and Tinopal C1101 are very effective to protect *Lymantria dispar* Nucleopolyhedrovirus –LdNPV-, *Helicoverpa zea* Nucleopolyhedrovirus –HzNPV-, and *Spodoptera frugiperda* Multiple Nucleopolyhedrovirus –SfMNPV- (Shapiro *et al*., 1994; Zou & Young, 1994, 1996; Mondragón *et al*., 2007). Recent studies have shown that the addition of 1% (wt:vol) aqueous extracts of cocoa (*Theobroma cacao* L.) (Malvales: Malvaceae), coffee (*Coffea arabica* L.) (Gentianales: Rubiaceae), and green and black tea (*Camellia sinensis* L.) (Ericales: Theaceae) provided excellent UV radiation protection for the beet armyworm, *Spodoptera exigua* Multiple Nucleopolyhedrovirus under laboratory conditions (SeMNPV) (El-Salamouny *et al*., 2009).

Regarding the safety of genetically modified baculovirus, a recombinant HzNPV carrying the *aait* gene was not pathogenic for bees, birds, fish, and other vertebrates (Szewczyk *et al*., 2006). Natural enemies of the larvae, like parasitoids and predators, were not adversely affected by ingesting individuals infected with recombinant viruses (Li *et al*., 1999; Smith *et al*., 2000; Boughton *et al*., 2003). So far, neither has been shown that the transgene be

transferred from baculovirus to other organism or virus (Inceoglu *et al*., 2001). On this basis, there is no evidence that a recombinant baculovirus is more dangerous than the corresponding wild type.

Finally, knowledge about the biology of baculoviruses suggests that bioinsecticides based on formulations containing these viruses have much lower risk to the environment than the classic chemical insecticides (Szewczyk *et al*., 2006).

5. Acknowledgment

This work was supported by research funds from *Agencia Nacional de Promoción Científica y Técnica* (ANPCyT) and *Universidad Nacional de Quilmes*. PDG is member of the Research Career of CONICET (*Consejo Nacional de Ciencia y Tecnología*); MNB holds a postdoctoral fellowship of CONICET.

6. References

Aranda, E.; Sánchez, J.; Peferoen, M.; Güereca, L.; Bravo, A. (1996). "Interactions of the *Bacillus thuringiensis* crystal proteins with the midgut epithelial cells of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)". J. Invertbr. Pathol. 68, pp. 203-212.

Barbosa, A.E.A.D.; Albuquerque1, E.V.S.; Silva1, M.C.M.; Souza1, D.S.L.; Oliveira-Neto1, O.B.; Valencia, A.; Rocha1, T.L.; Grossi-de-Sa, M.F. (2010). "α-amylases inhibitor-1 gene from *Phaseolus vulgaris* expressed in *Coffea arabica* plants inhibits α-amylases from the coffee berry borer pest". BMC Biotechnology. 10, pp. 44-52.

Barsoum, J.; Brown, R.; McKee, M.; Boyce, F.M. (1997). "Efficient transduction of mammalian cells by a recombinant baculovirus having the vesicular stomatitis virus G glycoprotein". Hum. Gene Ther., 8, pp. 2011-2018.

Bird, F.T.; Burk, J.M. (1961). "Artificial disseminated virus as a factor controlling the European spruce sawfly, *Diprion hercyniar* (Htg) in the absence of introduced parasites". Canad. Entomol. 96, pp. 228-238.

Black, B.C.; Brennan, L.A.; Dierks, P.M.; Gard, I.E. (1997). "Commercialization of baculoviral insecticides". In The baculovirus, ed by Miller LK, Plenum Press, New York, pp. 341-387.

Boughton, A.J., Obrycki, J.J.; Bonning, B.C. (2003). "Effects of a protease-expressing recombinant baculovirus on nontarget insect predators of *Heliothis virescens*". Biol. Control. 28, pp. 101-110.

Brown, W.E.; Takio, K.; Titani, K.; Ryan, C.A. (1985). "Wound-induced trypsin inhibitor in alfalfa leaves: identity as a member of the Bowman-Birk inhibitor family". Biochemistry 24, pp. 2195-2108.

Caballero, P.; Murillo, R.; Muñoz, D.; Williams, T. (2009). "El nucleopoliedrovirus de *Spodoptera exigua* (Lepidoptera: Noctuidae) como bioplaguicida: análisis de avances recientes en España". Revista Colombiana de Entomología 35, pp. 105-115.

Chakrabarty, S.; Monsour, C.; Teakle, R.; Reid, S. (1999). "Yield, biological activity, and field performance of a wild-type Helicoverpa nucleopolyhedrovirus produced in H. zea cell cultures". J. Invertebr. Pathol. 73, pp. 199-205.

Cherry, A.; Williams, T. (2001). "Control de insectos plaga mediante los baculovirus". En: Los baculovirus y sus aplicaciones como bioinsecticidas en el control biológico de

plagas. P. Caballero, M. López-Ferber. & T. Williams Eds. Phytoma S.A., Valencia, España. pp. 389-450.

Coelho, J.S.; Santos, N.D.; Napoleão, T.H.; Gomes, F.S.; Ferreira, R.S.; Zingali, R.B.; Coelho, L.C.; Leite, S.P.; Navarro, D.M.; Paiva, P.M. (2009). "Effect of *Moringa oleifera* lectin on development and mortality of *Aedes aegypti* larvae". Chemosphere 77, pp. 934-938.

Cory, J.S.; Hirst, M.L.; Williams, T.; Halls, R.S.; Goulson, D.; Green, B.M.; Carty, T.M.; Possee, R.D.; Cayley, P.J.; Bishop, D.H.L. (1994). "Field trial of a genetically improved baculovirus insecticide". Nature (London). 360, pp. 138-140.

Crickmore, N.; Zeigler, D.; Feitelson, J.; Schnepf, E.; Van Rie, J.; Lereclus D. (2008). "*Bacillus thuringiensis* toxin database". Online: http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/index.html.

Dawson, G.W.; Hallahan, D.L., Mudd, A.; Patel, M.M.; Pickett, J.A.; Wadhams, L.J.; Wallsgrove, R.M. (1989). "Secondary plant metabolites as targets for genetic modification of crop plants for pest resistance". Pestic. Sci. 27. pp 191-201.

Down, R.E.; Fitches, E.C.; Wiles, D.P.; Corti, P.; Bell, H.A.; Gatehouse, J.A. et al.. (2006). "Insecticidal spider venom toxin fused to snowdrop lectin is toxicto the peach-potato aphid, *Myzus persicae* (Hemiptera: *Aphididae*) and the rice brown planthopper, *Nilaparvata lugens* (Hemiptera:Delphacidae)". Pest Manag. Sci. 62, pp. 77–85.

Elazar, M., Levi, R.; Zlotkin, E.J. (2001). "Targeting of an expressed neurotoxin by its recombinant baculovirus". Exp. Biol. 204, pp. 2637-2645.

El-Salamouny, S.; Ranwala, D.; Shapiro, M.; Shepard, B.M.; Farrar, R.R. Jr. (2009). "Tea, coffee, and cocoa as ultraviolet radiation protectants for the beet armyworm nucleopolyhedrovirus". J. Econ. Entomol. 102, pp. 1767-1773.

Elvira, S.; Williams, T.; Caballero, P. (2010). "Juvenile hormone analog technology: effects on larval cannibalism and the production of *Spodoptera exigua* (Lepidoptera: Noctuidae) nucleopolyhedrovirus". J. Econ. Entomol. 103, pp. 577-582.

EPA (Environmental Protection Agency) (2008). "Pesticide Registration eligibility decision (RED): *Bacillus thuringiensis*. Online: http://www.epa.gov/oppsrrd1/REDs/0247.pdf.

Evans, H.F.; Harrap, K.A. (1982). "Persistence of insect viruses". In Virus persistence. Vol 33, ed. BWJ Mahy, AC Minson & GK Darby. SGM Symposium Cambridge University Press, pp. 57-96.

Faulkner, P.; Kuzio, J.; Williams, G.V.; Wilson, J.A. (1997). "Analysis of p74, a PDV envelope protein of *Autographa californica* nucleopolyhedrovirus required for occlusion body infectivity in vivo". J. Gen. Virol. 78, pp. 3091-3100.

Feitelson, J.S.; Payne, J.; Kim, L. (1992). "*Bacillus thuringiensis*: insects and beyond". Nat. Biotech. 10, pp. 271-275.

Flint, M.L.; van den Bosch, R. (1981). "Introduction to Integrated Pest Management". Plenum Press. Nueva York, EEUU, pp. 240.

Fitches, E.C.; Bell, H.A.; Powell, M.E.; Back, E.; Sargiotti, C.; Weaver, R.J.; Gatehouse, J.A. (2010). "Insecticidal activity of scorpion toxin (ButaIT) and snowdrop lectin (GNA) containing fusion proteins towards pest species of different orders". Pest Manag. Sci. 66, pp. 74–83.

Fitches, E.; Edwards, M.G.; Mee, C.; Grishin, E.; Gatehouse, A.M.R.; Edwards, J.P.; Gatehouse, J.A. (2004). "Fusion proteins containing insect-specific toxins as pest control agents: snowdrop lectin delivers fused insecticidal spider venom toxin to insect haemolymph following oral ingestion". J. Insect Physiol. 50, pp. 61–71.

Franco, O.L.; Rigden, D.J.; Melo, F.R.; Grossi-de-Sá, M.F. (2002). "Plant α-amylase inhibitors and their interaction with insect α-amylases. Structure, function and potential crop protection". Eur. J. Biochem. 269, pp. 397-412.

Gatehouse, J.A.; Hilder, V.A.; Gatehouse, A.M.R. (1991). "Genetic engineering of plant for insect resistance". In Plant Genetic Engineering, Plant Biotechnology Series, Vol 1, ed. D. Grierson. Blackie & Sons Ltd, London/Chapman and Hall, New York, pp. 105-135.

Gatehouse, J.A. (2011). "Prospects for using proteinase inhibitors to protect transgenic plants against attack by herbivorous insects". Curr. Protein Pept. Sci. 12, pp. 409-416.

Gazit, E.; La Rocca, P.; Samson, M.S.; Shai, Y. (1998). "The structure and organization within the membrane of the helices composing the pore-forming domain of *Bacillus thuringiensis* δ-endotoxin are consistent with an "umbrella-like" structure of the pore". Proc. Natl. Acad. Sci. USA 95, pp. 12289-12294.

Georghiou, G.P.; Wirth, M.C. (1997). "Influence of exposure to single versus multiple toxins of *Bacillus thuringiensis* subsp. israelensis on development of resistance in the mosquito *Culex quinquefasciatus* (Diptera: Culicidae)". Appl. Environ. Microbiol. 63, pp. 1095-1101.

Gerber, D.; Shai, Y. (2000). "Insertion and organization within membranes of the δ-endotoxin pore-forming domain, helix 4-loop-helix 5, and inhibition of its activity by a mutant helix 4 peptide". J. Biol. Chem. 275, pp. 23602-23607.

Gong, L.; Yang, X.; Zhang, B.; Zhong, G.; Hu, M.Y. (2011). "Silencing of Rieske iron–sulfur protein using chemically synthesised siRNA as a potential biopesticide against *Plutella xylostella*". Pest Manag. Sci. DOI 10.1002/ps.2086

Gordon, D.; Moskowitz, H.; Eitan, M.; Warner, C.; Catterall, W.A.; Zlotkin, E. (1992). "Localization of receptor sites for insect-selective toxins on sodium channels by site-directed antibodies". Biochemistry 31, pp. 7622-7628.

Gordon, K.H.; Waterhouse, P.M. (2007). "RNAi for insect-proof plants". Nature Biotechnol. 25, pp. 1231-1232.

Graham, J.; McNicol, R.J.; Greig, K. (1995). "Towards genetic based insect resistance in strawberry using the cowpea trypsin inhibitor". Ann. Appl. Biol. 127, pp. 163-173.

Gramkow, A.W.; Perecmanis, S.; Sousa, R.L.; Noronha, E.F.; Felix, C.R.; Nagata, T.; Ribeiro, B.M. (2010). "Insecticidal activity of two proteases against *Spodoptera frugiperda* larvae infected with recombinant baculoviruses". Virol. J. 7, doi:10.1186/1743-422X-7-143.

Granados, R.R.; Federici, B.A. (1986). "Biology of baculoviruses". CRC, Boca Ratón, Florida.

Granados, R.R.; Lawler, K.A. (1981). "In vivo pathway of *Autographa californica* baculovirus invasión and infection". Virology 108, pp. 297-308.

Green, T.R. & Ryan, C.A. (1972). "Wound-induced proteinase inhibitor in plant leaves: a possible defence mechanism against insects". Science 175, pp. 776-777.

Grossi-de-Sá, M.F.; Chrispeels, M.J. (1997). "Molecular cloning of bruchid (*Zabrotes subfasciatus*) α-amylase cDNA and interaction of the expressed enzyme with bean α-amylase inhibitors". Insect Biochem. Mol. Biol. 27, pp. 271-281.

Haas-Stapleton, E.J.; Washburn, J.O.; Volkman, L.E. (2004). "P74 mediates specific binding of *Autographa californica* M nucleopolyhedrovirus occlusion-derived virus to primary cellular targets in the midgut epithelia of *Heliothis virescens* Larvae". J. Virol. 78, pp. 6786-6791.

Hajos, J.P.; Pijnenburg, J.; Usmany, M.; Zuidema, D.; Zavodszki, P.; Vlak, J.M. (2000). "High frequency recombination between homologous baculoviruses in cell culture". Arch. Virol. 145, pp. 159-164.

Hallahan, D.L., Pickett, J.A., Wadhams, L.J., Wallsgrove, R.M.; Woodcock, C.M. (1992). "Potential of secondary metabolites in genetic engineering of crops for resistance". In Plant genetic manipulation for crop protection-Biotechnology in agriculture No 7, ed. A.M.R. Gatehuse, V.A. Hilder and D. Boulter. CAB international, Wallingford, Oxon, UK, pp. 215-248.

Hamshou, M.; Van Damme, E.J.; Smagghe, G. (2010). "Entomotoxic effects of fungal lectin from *Rhizoctonia solani* towards *Spodoptera littoralis*". Fungal Biol. 114, pp. 34-40.

Harrap, K.A. (1970). "Cell infection by a nuclear polyhedrosis virus". Virology 42, pp. 311-318.

Hawtin, R.E.; Arnold, K.; Ayres, M.D.; Zanotto, P.M.; Howard, S.C.; Gooday, G.A.; Chappell, L.H.; Kitts, P.A.; King, L.A.; Posse, R.D. (1995). "Identification and preliminary characterization of a chitinase gene in the *Autographa californica* nuclear polyhedrosis virus genome". Virology 212, pp. 673-685.

Hoover, K.; Kishida, K.T.; DiGiorgio, L.A.; Workman, J.; Alaniz, S.A.; Hammon, B.D.; Duffey, S.S. (1998). "Inhibition of baculoviral disease by plant-mediated peroxidase activity and free radical generation". J. Chem. Ecol. 24, pp. 1949-2001.

Ignacimuthu, S.; Prakash, S. (2006). "Agrobacterium-mediated transformation of chickpea with alpha-amylase inhibitor gene for insect resistance". Journal of Biosciences 31, pp. 339–345.

Ignoffo, C.M. (1992). "Environmental factors affecting persistence of entomolpathogens". Florida Entomol. 75, pp. 516-525.

Inceoglu, A.B.; Kamita, S.G.; Hinton, A.C.; Huang, Q.; Severson, T.F.; Kang, K.; Hammock, B.D. (2001). "Recombinant baculoviruses for insect control". Pest Manag. Sci. 57, pp. 981-987.

Ives, A.R.; Glaum, P.R.; Ziebarth, N.L.; Andow, D.A. (2011). "The evolution of resistance to two-toxin pyramid transgenic crops". Ecol. Appl. 21, pp. 503-515.

James, C. (2011). "Global Status of Commercialized Biotech/GM Crops: 2010". Ithaca, NY, ISAAA www.isaaa.org.

Jehle, J.A.; Blissard, G.W.; Bonning, V.; Cory, J.S.; Herniou, E.A.; Rohrmann, G.F.; Theilmann, D.A.; Thiem, S.M.; Vlak, J.M. (2006). "On the classification and nomenclature of baculoviruses: A proposal for revision". Arch. Virol. 151, pp. 1257-1266.

Jiexian Jiang, Aiping Zeng, Xiangyun Ji, Nianfeng Wan and Xiaoqin Chen. (2011). "Combined effect of nucleopolyhedrovirus and *Microplitis pallidipes* for the control of the beet armyworm, *Spodoptera exigua*". Pest Manag. Sci. 67, pp. 705-713.

Kaur, M.; Singh, K.; Rup, P.J.; Kamboj, S.S.; Singh, J.J. (2009). "Anti-insect potential of lectins from Arisaema species towards *Bactrocera cucurbitae*". Environ. Biol. 30, pp. 1019-1023.

Kuzio, J.; Jaques, R.; Faulkner, P. (1989). "Identification of p74 a gene essential for virulence of baculovirus occlusion bodies". Virology 173, pp. 759-763.

Lee, Y.; Fuxa, J.R.; Inceoglu, A.B.; Alaniz, S.A.; Richter, A.R.; Reilly, L.M.; Hammock, B.D. (2001). "Competition between wild type and recombinant nucleopolyhedroviruses in a greenhouse microcosm". Biol. Control 20, pp. 84-93.

Maeda, S. (1989). "Increased insecticidal effect by a recombinant baculovirus carrying a synthetic diuretic hormone gene". Biochem. Biophys. Res. Commun. 165, pp. 1177-1183.

Mettenmeyer, A. (2002). "Viral insecticidas hold promise for bio-control". Farming Ahead 124, pp. 50-51.

Mondragón, G.; Pineda, S.; Martínez, A.; Martínez, A.M. (2007). "Optical brightener Tinopal C1101 as an ultraviolet protectant for a nucleopolyhedrovirus". Commun. Agric. Appl. Biol. 72, pp. 543-547.

Moscardi, F. (1989). "Use of viruses for pest control in Brazil: the case of the nuclear polyhedrosis virus of the soybean caterpillar, *Anticarsia gemmatalis*". Memorias do Instituto Oswakdo Cruz, Rio de Janeiro, 84, pp. 51-56.

Moscardi, F. (1999). "Assessment of the application of baculoviruses for control of Lepidoptera". Annu. Rev. Entomol. 44, pp. 257-289.

Neppl, C. (2000). "Management of resistance to *Bacillus thuringiensis* toxins". The Environmental Studies Program Education- University of Chicago (Chicago, U.S.A.).

Norris, D.M. & Kogan, M. (1980). "Biochemical and morphological bases of resistance". In Breeding Plants Resistant to Insects, ed. F.G. Maxwell and P.R. Jennings. John Wiley & Sons, London, pp. 23-60.

O´Reilly, D.R.; Miller, L.K. (1991). "A baculovirus blocks insecto molting by producing ecdysteroid UDP-Glucosyl Transferase". Science (Washington) 245, pp. 1110-1112.

Payan, F. (2004). "Structural basis for the inhibition of mammalian and insect α-amylases by plant protein inhibitors". Biochim. Biophys. Acta 1696, pp. 171-180.

Pelegrini, P.B.; Murad, A.M.; Grossi-de-Sa, M.F.; Mello, L.V.; Romeiro, L.A.; Noronha, E.F.; Caldas, R.A.; Franco, O.L. (2006). "Structure and enzyme properties of *Zabrotes subfasciatus* α-amylase". Arch. Insect Biochem. Physiol. 61, pp. 77-86

Popham, H.J.R.; Li, Y.; Miller, L.K. (1997). "Genetic improvement of *Helicoverpa zea* polyhedrosis virus as a biopesticide". Biol. Control 10, pp. 83-91.

Price, D.R.G.; Gatehouse, J.A. (2008). "RNAi-mediated crop protection against insects". Trends Biotechnol. 26, pp. 393-400.

Pritchett, D.W.; Young, S.Y.; Geren, C.R. (1981). "Proteolytic activity in the digestive fluid of larvae of *Trichoplusia ni*". Insect Biochem. 11, pp. 523-526.

Pritchett, D.W.; Young, S.Y.; Yearian, W.C. (1984). "Some factors involved in the dissolution of *Autographa californica* NPV by digestive fluids of *Trichoplusia ni* larvae". J. Invert. Pathol. 43, pp. 160-168.

Pusztai, A.; Grant, G.; Steward, J.C.; Bardocz, S.; Ewen, S.W.B.; Gatehouse, A.M.R. (1992). "Nutritional evaluation of the trypsin inhibitor from cowpea". British J. Nutr. 68, pp. 783-791.

Regev, A.; Rivkin, H.; Inceoglu, B.; Gershburg, E.; Hammock, B.D.; Gurevitz, M.; Chejanovsky, N. (2003). "Further enhancement of baculovirus insecticidal efficacy with scorpion toxins that interact cooperatively". FEBS Lett. 537, pp. 106-110.

Rohrmann, G. (2008). "Baculovirus Molecular Biology". Bethesda (MD): National Library of Medicine (US), NCBI.

Sarmah, B.K.; Moore, A.; Tate, W.; Molvig, L.; Morton, R.L.; Rees, D.P.; Chiaiese, P.; Chrispeels, M.J.; Tabe, L.M.; Higgins, T.J.V. (2004). "Transgenic chickpea seeds expressing high levels of a bean alpha-amylase inhibitor". Mol. Breed. 14, pp. 73–82.

Schnepf, E.; Crickmore, N.; Van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J.; Zeigler, D.R.; Dean, D.H. (1998). "*Bacillus thuringiensis* and its pesticidal crystal proteins". Microbiol. Mol. Biol. 62, pp. 775-806.

Schroeder, H.E.; Gollasch, S.; Moore, A.; Tabe, L.M.; Craig, S.; Hardie, D.C.; Chrispeels, M.J.; Spencer, D.; Higgins, T.J.V. (1995). "Bean alpha-Amylase lnhibitor Confers Resistance to the Pea Weevil (*Bruchus pisorum*) in Transgenic Peas (*Pisum sativum*)". Plant Physiol. 107, pp. 1233–1231. 1239.

Sedlak, M.; Walter, T; Aronson, A. (2000). "Regulation by overlapping promoters of the rate of synthesis and deposition into crystalline inclusions of *Bacillus thuringiensis* δ-endotoxins". J. Bacteriol. 182, pp. 734-741.

Shade, R.E.; Schroeder, H.E.; Pueyo, J.J.; Tabe, L.M.; Murdock, L.L.; Higgins, T.J.V.; Chrispeels, M.J. (1994). "Transgenic Pea-seeds expressing the alpha-amylase inhibitor of the common bean are resistant to bruchid beetles". Bio-Technology 12, pp. 793–796.

Shapiro, M.; Daugherty, E.M. (1994). "Enhacement in activity of homologous and heterologous viruses against gypsy moth (Lepidoptera, Lymantridae) by an optical brightener". J. Econ. Entomol. 87, pp. 361-365.

Shumway, L.K.; Yang, V.V.; Ryan, C.A. (1976). "Evidence for the presence of proteinase inhibitors I in vacuolar protein bodies of plant cell". Planta 129, pp. 161-165.

Slack, J.M.; Kuzio, J.; Faulkner, P. (1995). "Characterization of v-cath, a cathepsin, L-like proteinase expressed by the baculovirus *Autographa californica* multiple nuclear polyhedrosis virus". J. Gen. Virol. 76, pp. 1091-1098.

Smith, C.R.; Heinz, K.M.; Sansone, C.G.; Flexner, J.L. (2000). "Impact of recombinant baculovirus field applications on non-target heliothine parasitoid *Microplitis croce*ipes (Hymenoptera: Braconidae)". J. Econ. Entomol. 93, pp. 1109-1117.

Solleti, S.K.; Bakshi, S.; Purkayastha, J.; Panda, S.K.; Sahoo, L. (2008). "Transgenic cowpea (*Vigna unguiculata*) seeds expressing a bean alpha-amylase inhibitor 1 confer resistance to storage pests, bruchid beetles". Plant Cell Rep. 27, pp. 1841–1850.

Sun, X.; Sun, X.; van der Werf, W.; Vlak, J.M.; Hu, Z. (2004). "Field inactivation of wild type and genetically modified *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus in cotton". Biocontrol Sci. Technol. 14, pp. 185-192.

Szewczyk, B., Hoyos-Carvajal, L.; Paluszek, M,; Skrzecz, I.; Lobo de Souza, M. (2006). "Baculoviruses-re-emerging biopesticides". Biotechnol. Adv. 24, pp. 143-160.

Walker-Simmons, M. & Ryan, C.A. (1977). "Immunological identification of proteinase inhibitor I and II in isolated tomato leaf vacuoles". Plant Physiol. 60, pp. 61-63.

Washburn, J.O.; Chan, E.Y.; Volkman, L.E.; Aumiller, J.J.; Jarvis, D.L. (2003). "Early synthesis of budded virus envelope fusion protein GP64 enhances *Autographa californica* multicapsid nucleopolyhedrovirus virulence in orally infected *Heliothis virescens*". J. Virol. 77, pp. 280-290.

Whyard, S.; Singh, A.D.; Wong, S. (2009). "Ingested double-stranded RNAs can act as species-specific insecticides". Insect Biochem. Mol. Biol. 39, pp. 824-832.

Wudayagiri, R.; Inceoglu, B.; Herrmann, R.; Derbel, M.; Choudary, P.V.; Hammock, B.D. (2001). "Isolation and characterization of a novel lepidopteran-selective toxin from the venom of South Indian red scorpion, *Mesobuthus tamulus*". BMC Biochem. 2, pp. 11-16.

Xu, D.P.; Xue, Q.Z.; McElroy, D.; Mawal, Y.; Hilder, V.A.; Wu, R. (1996). "Constitutive expression of a cowpea trypsin-inhibitor gene, CpTI, in transgenic rice plants confers resistance of two major rice insect pest". Mol. Breed. 2, pp. 167-173.

Yamada, T.; Hattori, K.; Ishimoto, M. (2001). "Purification and characterization of two α-amylase inhibitors from seeds of tepary bean (*Phaseolus acutifolius* A. Gray)". Phytochem. 58, pp. 59-66.

Yang, Z.; Chen, H.; Tang, W.; Hua, H.; Lin, Y. (2011). "Development and characterisation of transgenic rice expressing two *Bacillus thuringiensis* genes". Pest Manag. Sci. 67, pp. 414-422.

Yu, H.L.; Li, Y.H.; Wu, K.M. (2011). "Risk Assessment and Ecological Effects of Transgenic *Bacillus thuringiensis* Crops on Non-Target Organisms(F)". J. Integr. Plant Biol. 53, pp. 520-538.

Zhang, G.Y.; Sun, X.L.; Zhang, Z.X.; Zhang, Z.F.; Wan, F.F. (1995). "Production and effectiveness of the new formulation of Helicoverpa virus pesticide-emulsifiable suspension". Virologica Sinica 10, pp. 242-247.

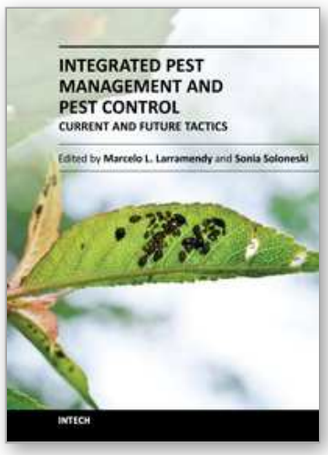
Zhou, M.Z.; Sun, H.C.; Hu, Z.H.; Sun, X.L. (2004). "SOD enhances infectivity of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrosis virus against *H. armigera* larvae". Virologica Sinica 18, pp. 506-507.

Zhou, X.Y.; Liu, N.; Zhao, M.; Li, H.; Zhou, L.; Tang, Z.W.; Cao, F.; Li, W. (2011). "Advances in effects of insecticidal crystal proteins released from transgenic Bt crops on soil ecology". Yi Chuan. 33, pp. 443-448.

Zhu, F.; Xu, J.; Palli, R.; Ferguson, J.; Palli, S.R. (2011). "Ingested RNA interference for managing the populations of the Colorado potato beetle, *Leptinotarsa decemlineata*". Pest Manag. Sci. 67, pp. 175-182.

Zou, Y.; Young, Y.S. (1994). "Enhancement of nuclear polyhedrosis virus activity in larval pest of Lepidoptera by a stilbene fluorescent brightener". J. Entomol. Sci. 29, pp. 130-133.

Zou, Y.; Young, Y.S. (1996). "Use of fluorescent brightener to improve *Pseudoplusia includens* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus activity in the laboratory and field". J. Econ. Entomol. 89, pp. 92-96.



Integrated Pest Management and Pest Control - Current and Future Tactics

Edited by Dr. Sonia Soloneski

ISBN 978-953-51-0050-8
Hard cover, 668 pages
Publisher InTech
Published online 24, February, 2012
Published in print edition February, 2012

Integrated Pest Management is an effective and environmentally sensitive approach that relies on a combination of common-sense practices. Its programs use current and comprehensive information on the life cycles of pests and their interactions with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means and with the least possible hazard to people, property, and the environment.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Vanina Andréa Rodriguez, Mariano Nicolás Belaich and Pablo Daniel Ghiringhelli (2012). Baculoviruses: Members of Integrated Pest Management Strategies, Integrated Pest Management and Pest Control - Current and Future Tactics, Dr. Sonia Soloneski (Ed.), ISBN: 978-953-51-0050-8, InTech, Available from: http://www.intechopen.com/books/integrated-pest-management-and-pest-control-current-and-future-tactics/baculoviruses-members-of-integrated-pest-management-strategies

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen