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BACULOVIRUS BIOPESTICIDES: AN OVERVIEW

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ABSTRACT

Baculoviruses have shown to be a good tool for insect pest control. Numerous natural baculoviruses have been used as biopesticides worldwide as they are naturally occurring pathogens, highly specific with limited host range, and without lethal effects on non-target organisms. Slow speed of kill of wild-type baculoviruses when compared to chemical insecticides is a serious disadvantage that has promoted the development of large number of recombinant baculoviruses, mainly with genes encoding insect-specific toxins from scorpions or spiders, which kill insects faster than the parent wild-type virus. However, uptill today no recombinant baculovirus-based pesticides are commercially available. For the expansion of baculovirus pesticides some drawbacks have to be overcome. Current methods for large scale *in vitro* production have to be improved in order to circumvent their technical limitations, and developments of new and more effective additives to improve the stability of baculoviruses in pesticide formulation are needed. Nonetheless, recombinant baculoviruses are expected to strongly contribute to the expansion of baculovirus pesticides use in the next future, as the negative social perception of genetically modified organisms, including genetically modified baculoviruses, decreases.

Key words: Baculovirus; biopesticide; recombinant; production; formulation

INTRODUCTION

To date, the most common method for pest control has been the classical organic pesticides. These pesticides were first introduced in the 1940s with the use of dichloro-diphenyl-trichloroethane (DDT), followed by other organophosphate and carbamate pesticides introduced in the 1960s (Nicholson, 2007).

Although chemical pesticides have many advantages, there are widely held concerns over their indiscriminate use. These concerns include the contamination of land, air and water, the contribution to the reduction of the ozone layer and to global warming, development of resistance in target pests, detrimental effects in non-target organisms, and impact on human health (Casida and Quistad, 1998; Fitches *et al.*, 2010; Al-Zaidi *et al.*, 2011).

Nowadays, the availability of many of the older, broad-spectrum chemical pesticides is declining as a result of the evolution of resistances (Ishtiaq *et al.*, 2012) and legislation (Chandler *et al.*, 2011). Therefore, alternative, environmentally safe methods are needed (O'Callaghan and Brownbridge, 2009).

Different biopesticides, such as viruses, microorganisms (bacteria, fungi, etc.), microorganism derived products, animal derived products (pheromones, hormones, insect-specific toxins, etc.), plant derived products or genetically modified organisms have been developed and used across the world (Nicholson, 2007; Erlandson, 2008; Mazhabi *et al.*, 2011; Islam and Omar, 2012).

Baculoviruses biopesticides have many advantages as tool in integrated pest management (IPM), including highly specificity, safe to vertebrates and plants, and ease of genetic manipulation. However, baculoviruses, like others biopesticides, present some difficulties for broad commercial use, such as slow speed of kill, short field stability, high production costs, and current regulations of biological control agents (Mills and Kean, 2010; Ravensberg, 2011; Regnault-Roger, 2012). In this paper we provide and overview of currently available baculovirus biopesticides technology.

Baculovirus biology: Baculoviruses are the major group of arthropod viruses (Herniou and Jehle, 2007). They are the most intensely studied insect pathogenic viruses (Inceoglu *et al.*, 2006), and have been used for biological control of forestry and agriculture pests since 1900s. Currently, they are also used as expression vectors in biotechnology (van Oers, 2011; Contreras-Gómez *et al.*, 2014). Although most of the baculovirus have been isolated from the order Lepidoptera, where around 500 species are known to be hosts, they have also been isolated from other insect orders such as Neuroptera, Trichoptera, Crustacea, Hymenoptera, Coleoptera, and Diptera (Possee *et al.*, 1997; Szewczyk *et al.*, 2006).

There are two phenotypes of baculoviruses: the budded virus (BV) and the occluded virus (OV) (Jehle *et al.*, 2006). BVs are produced after initial infection and budding through the plasma membrane of infected cells. BV particles serve to transmit the virus to other tissues within an infected insect. OVs are produced and occluded in a crystalline protein matrix called occlusion body (OB) in the late stage of infection. Baculoviruses occlude their nucleocapsids in polyhedral shaped OBs that may carry over 100 virions (nucleopolyhedroviruses, NPVs, genera *Alphabaculovirus* –specific for lepidopteran–, *Gammabaculovirus* –specific for hymenopteran–, and *Deltabaculovirus* –specific for dipteran–) or in granular OBs carrying only one virion (granuloviruses, GV, genus *Betabaculovirus* –specific for lepidopteran–) (Jehle *et al.*, 2006). Both of them are specific to the larval stage of their insect hosts (Inceoglu *et al.*, 2001). OBs are responsible for the spread of the virus amongst insects, mainly by larvae ingestion of OBs present on plants (Szewczyk *et al.*, 2006). OBs are solubilized in the midgut and the released virions initiate infection of the midgut cells. The OBs will be later spread by excrements of infected insects (Vasconcelos, 1996).

The NPVs are phylogenetically subdivided into two groups, type I and type II. The main difference between them is that type I NPVs contain the fusion protein GP64, needed for virus entry into the cell and for cell-to-cell transmission, while type II NPVs lack GP64 and instead contain a generic fusion protein, called F protein, with the same role as GP64. This protein can also be found in some vertebrate viruses (Szewczyk *et al.*, 2006). In alphabaculoviruses, some of NPV virions have a single nucleocapsid (SNPV), while others have multiple nucleocapsids (MNPV), depending on the virus species (Possee *et al.*, 1997), although this trait has no taxonomic meaning.

Baculoviruses as insecticides: As stated above, natural baculoviruses have been used for pests control in forestry and for the production of orchard and field crops since the early years of the last century worldwide (Inceoglu *et al.* 2006). The use of baculoviruses as insecticides presents several advantages in comparison with chemical insecticides (Possee *et al.*, 1997; Inceoglu *et al.*, 2006): They are natural pathogens, highly specific to insect and closely related arthropods, they are safe pathogenically to vertebrates and other beneficial organisms, they have capacity to persist in the environment, and they can be mass-produced, formulated, packaged, stored, and marketed in a similar way to chemical pesticides.

Although natural baculoviruses have been satisfactorily applied to protect crops and forests, they are imperfect insecticides from an agroindustrial point of view and present several potential limitations (Possee *et al.*, 1997; Inceoglu *et al.*, 2006): Slower speed of kill (ranging from five days to greater than two weeks) than chemical pesticides (hours), very narrow host specificity, short field stability, susceptibility to UV light, short shelf life, and high production costs compared with chemical insecticides.

Nonetheless, baculoviruses are ideal tools in IPM programs and some of them, expecially those which have been widely studied and characterized, have had important field results. About 60 baculovirus-based pesticides have been utilized to control diverse insect pest all over the world. Examples of commercial baculovirus biopesticides are depicted in Table 1. In the following paragraphs, emphasis is made on the most important.

Anticarsia gemmatalis is a frequent and abundant soybean pest in Brazil that used to require frequent insecticide applications, until an IPM program was implemented in 1982, and AgMNPV was used to threat approximately 2000 ha of soybean (Moscardi, 1999). The treated areas increased up to two million ha by 2002-2003 (Szewczyk *et al.*, 2006). The use of AgMNPV to control *A. gemmatalis* in Brazil was a very successful program and has been considered as the most important one in the world (Yang *et al.*, 2012). Based on this program, AgMNPV use was extended to other South American countries (Sosa-Gómez *et al.*, 2008).

The codling moth *Cydia pomonella* is a worldwide pest of apple, pears and walnuts (Arthurs *et al.*, 2007). The isolation of the *C. pomonella* granulovirus (CpGV) in 1964 provided a highly effective pathogen for insect control; protecting fruit from economic damage in integrated pome fruit production in Europe and many other areas in several countries (Moscardi, 1999; Lacey *et al.*, 2008; Kutinkova *et al.*, 2012). CpGV is probably the most important viral biopesticide applied worldwide in terms of treated area (Vincent *et al.*, 2007). Nonetheless, since 2005, evidences of a reduction in susceptibility of *C. pomonella* to CpGV pesticides have been reported in Germany and France (Shulze-Bopp and Jehle, 2012; Undorf-Spahn *et al.*, 2012). An up to 10,000-fold increase in resistance in comparison to susceptible *C. pomonella* populations has been observed (Asser-Kaiser *et al.*, 2007). Many efforts are been made to find CpGV isolates effective against resistant *C. pomonella* colonies (Berling *et al.*, 2009).

The genera *Helicoverpa* and *Heliothis* represent a key pests worldwide, responsible for millions of dollars in losses every year (Moscardi *et al.*, 2011). *Helicoverpa armigera,* commonly known as cotton bollworm, is a highly polyphagous pest and one of the most serious pest around the world, attacking many crops such as cotton, pepper, maize, tomatoes, tobacco, sorghum, sunflower and several types of beans (Sun *et al.*, 2002). *H. armigera* is the noctuid specie possessing by far the most reported resistance cases to a wide range of pesticides worldwide (Joußen *et al.*, 2012; Mironidis *et al.*, 2013), including resistance to transgenic Bt cotton (Luttrell and Jackson, 2012; Yang *et al.*, 2013). One approach to counteract these resistances has been the use of baculovirus (Rohrmann, 2011). In laboratory experiments, mortalities up to almost 98% have been achieved (Figueiredo *et al.*, 2009; Elamathi *et al.*, 2012). In field trials, it has also been found that the combination of HaMNPV with endosulfan has given better results (Mir *et al.*, 2010; Siddique *et al.*, 2010). Locally isolates of HaSNPV have been produced and applied to different crops in Australia, India, and China (Moscardi *et al.*, 2011). In China HaSNPV is one of the most important commercial baculovirus. Different NPVs are applied on over 100,000 ha annually (Yang *et al.*, 2012). The corn earworm, *Helicoverpa zea*, and its related species, the tobacco budworm, *Hiliothis virescens* represent global pests that affect many fibre and food crops and, frequently, have developed resistance to most chemical pesticides (Hunter-Fujita *et al.*, 1998). The estimated cost of both species in the USA is estimated to be 1$ billion (López *et al.*, 2010). Their NPVs are effective against several species of the genera *Helicoverpa* and *Hiliothis* on cotton, corn, sorghum, soybeans, tobacco and tomato.

Another genus responsible of a drastic impact on food production is *Spodoptera*, including *S. exigua*, *S. frugiperda*, *S. littoralis* and *S. litura* (Moscardi *et al.*, 2011). *S. exigua*, also known as the beet armyworm, is a polyphagous and widely distributed pest on many vegetable, field and ornamental crops (Murillo *et al.*, 2006). Its baculovirus, SeMNPV, is specific and highly pathogenic in both the field and the laboratory. In the greenhouses of Almería, Spain, the largest extension of covered crops in Europe, *S. exigua* is a very important pest and has developed resistance to many chemical insecticides. In 2009, a field trial showed that the field efficacy of a simple formulation of SeMNPV pesticide was greater than that offered by treatments of various chemical insecticides (Caballero *et al.*, 2009). Additionally, the infectivity of SeMNPV can be enhanced by combined use with camptothecin (Sun *et al.*, 2012). The fall armyworm, *S. frugiperda*, is an important and widely distributed pest in South America, causing severe yield losses in corn, rice, and sorghum (Barrera *et al.*, 2011). Currently, there are several control programs under development in different countries (Barrera *et al.*, 2011; Martínez *et al.*, 2012). The cotton leaf worm *S. littoralis* is prevalent in Africa and the Mediterranean region, and is considered to be the most serious cotton pest in Egypt (Hatem *et al.*, 2011). In this country, the use of SliNPV has been very successful to control *S. littoralis* (Adel-Sattar *et al.*, 2012).

Defoliating larvae of Hymenoptera and Lepidoptera are important pests in forest systems (Moscardi *et al.*, 2011). *Lymantria dispar*, the gypsy moth, is an extremely important defoliator of broadleaved trees. In the USA, LdMNPV was considered a potential biocontrol agent in the early 1900s, and efforts to develop the baculovirus as a control agent for *L. dyspar* began in earnest in the 1960s (Solter and Hajek, 2009). Thereafter, the use of LdMNPV spread worldwide. From 2004 to 2006, the activity of three geographic isolates (Chinese, American and Japanese) of LdMNPV was evaluated in field trials in China. Results showed that Japanese isolate was significant more virulent than the other two (Duan *et al.*, 2012). Forest systems are ecologically diverse and more stable than agricultural systems, allowing baculoviruses to remain there for long periods of time (Moscardi *et al.*, 2011).

Recombinant baculoviruses: The slow speed of kill of wild-type baculoviruses prevents their practical use, and many strategies have been developed to improve their killing action by modifying the baculovirus using recombinant DNA technology, including the insertion of genes encoding insect hormones or enzymes, or insect-specific toxins (Kamita *et al.*, 2005; Li and Bonning, 2007; Gramkow *et al.*, 2010).

In 1989, Maeda was the first to successfully generate a genetically modified baculovirus expressing a gene encoding a hormone (Maeda, 1989). This baculovirus expressed the gene of a diuretic hormone that caused larvae of *Bombyx mori* to lose water, disrupting the normal physiology of the insect. The speed of kill of this modified BmNPV was 20% faster than that of parent wild-type BmNPV. This work introduced a new concept and established the foundations for subsequent approaches to kill insects using baculoviruses.

In the following years, other enzymes and hormones were tried to modify baculoviruses. Juvenile hormone esterase (Hammock *et al.*, 1990), eclosion hormone (Eldridge *et al.*, 1991) and prothoracicotropic hormone (O´Reilly *et al.*, 1995) were successfully expressed in recombinant baculoviruses. However, only those expressing juvenile hormone esterase showed a significant improvement in insecticidal activity over parent wild-type baculovirus (El-Sheikh *et al.*, 2011a). Since juvenile hormone esterase regulates juvenile hormone, its overexpression produces a decrease in the concentration of the hormone. This leads to halt insect feeding and pupate (El-Sheikh *et al.*, 2011b). The short half-live of juvenile hormone esterase in the hemolymph is a serious constrain to the efficient use of recombinant baculoviruses expressing this enzyme. Nonetheless many efforts have been made to improve *in vivo* stability to make it more efficient (Hinton and Hammock, 2003; Inceoglu *et al.*, 2006; Kamita and Hammock, 2010).

Another approach to enhancing the speed of kill of the baculoviruses involves deleting an endogenous gene, such as the baculovirus-encoded enzyme ecdysteroid UDP-glucosyltransferase gene (O´Reilly and Miller, 1991). Since ecdysteroids are hormones that regulate larval-pupal molting and feeding activity, infection with an egt deletion mutant baculovirus results in a reduction in food consumption and an earlier mortality (Eldridge *et al.*, 1992; Wilson *et al.*, 2000; Cai *et al.*, 2010; Georgievska *et al.*, 2010).

Genetically modified baculoviruses expressing toxins have been historically much widely exploited. The first successful results of toxin genes introduction into baculoviruses were reported in the late 1980s (Carbonell *et al.*, 1988; Tomalski *et al.*, 1988, 1989; Ooi *et al.*, 1989). Since then, most of the works were mainly committed to the study of arthropod-specific toxins from mites, spiders or scorpions (see Inceoglu *et al.*, 2006 for a review). *Androctonus australis* insect-specific toxin (AaiT) was the first and the most promising insect toxin that has been successfully expressed in baculuviruses (MacCutchen *et al.*, 1991; Maeda *et al.*, 1991; Stewart *et al.*, 1991). An increase up to 40% was observed in the speed of kill of silkworm larvae, when a recombinant *Bombix mori* baculovirus (BmNPV) expressing AaiT was used, compared with parent wild-type baculovirus (Maeda *et al.*, 1991). In other study with a different baculovirus expressing AaiT it was observed that larvae of *Manduda sexta* were paralyzed many hours prior to death (MacCutchen *et al.*, 1991) increasing pesticidal efficacy. The efficiency of AaiT-expressing baculoviruses was further corroborated in field trials (Cory *et al.*, 1994; Sun *et al.*, 2002; Sun *et al.*, 2004).

Although AaiT has been considered as the best model peptide neurotoxin for improving the insecticidal activity of the baculoviruses and continued to be the subject of numerous studies (Inceoglu *et al.*, 2006; Sun *et al.*, 2009), other toxins from scorpions *Leiurus quinquestriatus quinquestriatus, Leiurus quinquestriatus hebraeus* and *Buthus martensi* (Kopeyan *et al.*, 1990; Zlotkin *et al.*, 1993; Moskowitz *et al.*, 1998; Froy *et al.*, 2000; Tang *et al.*, 2011), spiders *Agelenopsis aperta, Dighetia canities*, *Tegenaria agrestis* and *Araneus ventricosus* (Prikhodko *et al.*, 1996; Hughes *et al.*, 1997; Jung *et al.*, 2012), or straw itch mite *Pyemotes tritici* (Tomalski and Miller, 1991; Lu *et al.*, 1996; Burden *et al.*, 2000) are also sources of potent toxins that, expressed in baculovirus, are active against insect pest and could potentially be used as biopesticides.

The molecular target of most of these neurotoxins is the insect sodium channel (Cestele and Catterall, 2000; Casida and Durkin, 2013), which is also the major target of insecticides belonging to the pyrethroid class. However, their specific binding sites on the channel do not overlap, so there is a possibility of producing a synergistic effect, allowing both baculovirus expressing toxins and pyrethroids to be used simultaneously (McCutchen *et al.*, 1997).

The most recent approach is the expression of crystal protein gene from *Bacillus thuringiensis* in *Autographa californica*. This recombinant baculovirus has showed a high insecticidal activity against *Spodoptera exigua* and *Plutella xylostella*, compared to those of parent wild-type AcMNPV (Jung *et al.*, 2012; Shim *et al.*, 2013).

Recombinant baculovirus have shown potential for improved insect pest control. However, the ecological impact of using such viruses needs to be assessed. Several studies have concluded that baculoviruses are not infectious toward non-target organisms, including beneficial insect species and predators, and parasitoids of lepidopteran larvae (Boughton *et al.*, 2003; Sun *et al.*, 2009). Recombinant AcMNPV baculovirus expressing AaiT was not infectious to adherent mammalian cells (Hartig *et al.*, 1991) and recombinant HaSNPV expressing AaiT was not pathogenic in any way to fish, birds and other vertebrates (Sun *et al.*, 2002).

According to different studies in greenhouse and in the field, a recombinant baculovirus does not have any selective ecological advantages when compared to the wild-type baculovirus (Cory *et al.*, 1994; Black *et al.*, 1997; Lee *et al.*, 2001). Furthermore, there is a negative selection against recombinant baculoviruses such that they are quickly displaced by the wild-type (Georgievska *et al.*, 2010; Zwart *et al.*, 2010). It has also been speculated on the probability that the cloned gene could jump from the recombinant baculovirus to another organism. Although, in theory, this is possible, there are factors excluding or limiting the occurrence of this genetic recombination so that it has never been proven (Inceoglu *et al.*, 2001).

Taking into consideration the studies about their safety and, although other important factors must be optimized (e.g. virus persistence in the environment, genetic stability, etc), it can be concluded that benefits from recombinant baculoviruses exceed suspected undefined risks and are a potential agent to be used against crop pests instead of chemical pesticides.

Production and formulation of baculovirus based bioinsecticides: Nowadays, baculoviruses commercial-scale production is done exclusively *in vivo*, applying the baculovirus in the field and collecting the infected larvae when they are dead or, mainly growing the larvae in the laboratory, using a feed contaminated with the baculovirus. However, these methods present some drawbacks, as difficulty to scale up the production economically, potential contamination of viral preparation with microorganisms, insect proteins or insect cuticles, or the fact that the mass rearing of insects is labor intensive and adds greatly to the final cost of the product (Lasa *et al.*, 2007a, Elvira *et al.*, 2010; Vreysen *et al.*, 2010).

It is possible to produce baculoviruses in a more cost effective and controlled manner using *in vitro* insect cell cultures with bioreactors (Possee *et al.*, 1997; Ikonomou *et al.*, 2003). Insect cells can be grown easily as static cultures. However, when adaptation to suspension culture is attempted, especially on large scale, many problems arise. Insect cells are very sensitive to shear stress and may be easily damaged by agitation and/or aeration (Beas-Catena *et al.*, 2011). A variety of factors affect cell growth in suspension, such as medium formulation, medium composition, serum concentration, pH, osmotic pressure, oxygen tension and bioreactor hydrodynamics (Beas-Catena *et al.*, 2013a; Drugmand *et al.*, 2012).

Insect cells can be grown in almost all culture modes, from batch to perfusion. Nevertheless, since the baculovirus infection ultimately leads to the lysis of the cell, either the batch or fed-batch mode are preferred. Because baculovirus production is a lytic process, the most important variables for the optimization of batch process are the time of infection, the multiplicity of infection and the harvest time (Maranga *et al.*, 2004; Aucoin *et al*., 2010; Beas-Catena *et al.*, 2013b).

The success of the baculovirus *in vitro* production strongly depends, among other things, on the medium costs, the insect cell line, and the volumetric productivity (Marteijn *et al.*, 2003; Huynh *et al.*, 2012). Additionally, the genotypic variations accumulation in baculovirus by serial passage is a serious constraint for *in vitro* production (Moscardi *et al.*, 2011), since serial passage of the baculovirus in cell culture results in reduced occlusion and loss of virulence (Krell, 1996). Furthermore, media formulation can greatly affect biological activity of produced baculovirus (Pedrini *et al.*, 2006; Huynh *et al.*, 2012). In addition, recombinant baculovirus expressing an insect-toxin have reduced yield of virus compared with wild type (Inceoglu *et al.*, 2006).

Although baculoviruses may remain unchanged for many years when stored under appropriated conditions (Sireesha *et al.*, 2010), they are rapidly inactivated under field conditions by short and long wavelengths (254 – 320 nm) UV light (Jeyarani *et al.*, 2013), particularly in the UV-B range (280-320 nm) (Lasa *et al.*, 2007b). UV radiation induces molecular changes in DNA by fusing two adjacent thymine bases on a DNA strand through the formation of a cyclobutane ring between the bases, thus creating a block of normal DNA synthesis and a high rate of mutation; less frequently DNA strands might break (Hunter-Fujita *et al.*, 1998). The use of UV protectans has therefore been proposed to prevent baculovirus inactivation.

Several protectans have been successfully used, such as reflectants (materials both opaque and reflectant which are usually bright substances, such as metallic aluminium, aluminium oxide or titanium dioxide), general absorbents (black materials which absorb from the infrared through the visible to the UV wavelenghts, such as carbon and naphthalene black), selective absorbents (substances with strong absorbance in UV-B which are often wholly or largely transpartent to the visible wavelengths, such as amelozan and p-aminobenzoic acid), optical brighteners (substances that absorb UV radiation and emit light in the blue region of the spectrum, such as derivatives of stilbene, oxazole, pyrazole, naphthalic acid, lactone and coumarin), and chromophores (substances that transfer energy from highly absorbent and UV susceptible molecules -e.g. DNA- to energy-accepting molecules -or chromophores- before damage is significant, such as acriflavin, ethyl green, methyl green and rhodamine B) (Morales *et al.*, 2001; Martínez *et al.*, 2003; Lasa *et al.*, 2007b).

Several authors have tested the efficacy of UV protectant agents against inactivation of occluded viruses once released in the field, as well as the ability of these substances to enhance viral infection (Ibargutxi *et al.*, 2008; Lasa *et al.*, 2007b)

Plant metabolites which generate free radicals may also produce the inactivation of baculoviruses. This is minimized by adding free radicals scavengers such as mannitol (Zhou *et al.*, 2004). Developments as those discussed above, increase the effectiveness of baculovirus biopesticides formulations and may help to reduce the price and contribute to make them competitive with chemical pesticides in a next future.

Concluding remarks and future prospects: Although wild-type baculoviruses show slow speed of kill when compared to chemical pesticides, in some cases, such as forest ecosystems, they have shown to be a good option over the long term. Nonetheless, slow speed of kill is a serious disadvantage that has led to develop large number of recombinant baculovirus during the last 20-plus years, mainly with toxins genes from scorpions or spiders which kill insects faster than the parent wild-type virus. However, up to date, baculovirus biopesticides have a low market influence worldwide (about 0.5% of the total pesticides market according to Moscardi *et al.*, 2011), and no recombinant baculovirus is commercially available. Hopefully, in the next future, recombinant baculoviruses will progressively increase their presence in the global marker. However, for this to happen, some difficulties have to be overcome. One of the unresolved issues in baculovirus technology is the *in vitro* production on a commercial perspective, since all of about 60 baculovirus formulations commercially available are produced *in vivo*. Probably, the most important limitation for *in vitro* production of baculoviruses is the genotypic variations accumulation in cell culture, which prevents the production at large-scale. Furthermore, the expression of an insect-toxin reduces yield of virus and may have other effects on the final product. In the future, the methods for large-scale *in vitro* production have to be improved in order to circumvent these drawbacks, simplify commercial production and reduce the costs. Another shortcoming is baculovirus biopesticides formulation, since stability of baculoviruses is influenced by environmental factors such as pH, humidity, temperature, and mainly by ultraviolet light, which is highly detrimental to viral survival. Although several protectans have been successfully used, the best results have been obtained with several brighteners. Future developments of new and more effective brighteners will reduce the cost of baculoviruses insecticides. Nonetheless, the most relevant concern for the use of baculovirus insecticides will be the public acceptance and perception about the risks and benefits of recombinant baculoviruses to control insect pests. According with many reports, there is no proof that genetically modified baculoviruses have more risks to animals and the environment than the parent wild-type viruses (Szewczyk *et al.*, 2006; Inceoglu *et al.*, 2006; Moscardi *et al.*, 2011). Despite that, field trials of recombinant bauloviruses raised public protests that have delayed and slowed their use as pesticides. Clearly, the public needs better information on their risks and benefits, especially in comparison with chemical pesticides and, hopefully, the negative social perception will decrease. This will help to increase the use of recombinant baculoviruses and therefore to the expansion of baculovirus biopesticides.

Table 1: Main examples of commercial baculovirus biopesticides

Host insect	Baculovirus	Target crop(s)	Product name(s)	Key Reference
Adoxophyes orana	GV	Apple, pears,	Capex 2	Dickler (1991)
Agrotis segetum	GV	vegetables	Agrovir	Caballero *et al.* (1991)
Anticarsia gemmatalis	NPV	Soybean	Baculo-soja, Multigen, Baculovirus Nitral, Coopervirus SC, Protege	Moscadi (1999)
Autographa californica	NPV	Alfalfa, vegetables	Gusano Biological, VPN-80TM	Thakore *et al.* (2006)
Cydia pomonella	GV	Apple, pears, walnut	Cyd-X, Virosoft CP4, Madex, Granupom, Granusal, Carpovirusine, Virin-CyAp, Carposin, Carpovirus SC	Vincent *et al.* (2007)
Helicoverpa armigera	NPV	Cotton, pepper, soybean, tomato	Virin-HS, DOA BIO V2	Erlandson (2008)
Helicoverpa zea	NPV	Cotton, vegetables	GemStar, Biotrol, Elcar	Erlandson (2008)
Hyphantria cunea	NPV	Forestry	Virin-ABB	Moscardi (1999)
Lymantria dispar	NPV	Forestry	Disparvirus, Gypchek, Virin-ENSH,	Erlandson (2008)
Mamestra brassicae	NPV	Vegetables	Mamestrin, Virin EKS	Thakore *et al.* (2006)
Mamestra configurata	NPV	Oilseed rapes	Virosoft	Erlandson (2008)
Neodiprion lecontei	NPV	Forestry	Leconti-virus	Erlandson (2008)
Neodiprion sertifer	NPV	Forestry	Monisärmiövirus	Erlandson (2008)
Orgyia pseudotsugata	NPV	Forestry	Biocontrol I, Virtuss	Erlandson (2008)
Spodoptera albula	NPV	Horiculture	VPN-82, VPN-Ultra	Jackson *et al.* (2008)
Spodoptera exigua	NPV	Horticulture	Spod-X, Vir-ex, Spod-X LC, Otienem-STM, Ness-A, Ness-E, DOA BIO V1,	Erlandson (2008)
Spodoptera frugiperda	NPV	Corn, rice, maize, sorghum	-	Escribano *et al.* (1999)
Spodoptera littoralis	NPV	Cotton, corn	Spodopterin	Thakore *et al.* (2006)
Spodoptera litura	NPV	Tobacco, vegetables,	DOA BIO V3	Kamiya *et al.* (2004)

This list is not comprehensive. The reference is not necessarily the first time that the biopesticides were described.

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