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Naturally Occurring Baculoviruses for Insect Pest Control

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1. Introduction

Baculoviruses of insects have been promoted for their pest control potential for more than half a century ***(1)***. Despite this, only a few have been successful in biological control, and almost none has proven a commercial success, or is used routinely for large-scale insect control in industrialized countries. Baculoviruses have, however, achieved moderate success in some developing countries. Thus, in addition to discussing the use of naturally occurring baculoviruses as pest-control agents, the reasons why these viruses have not been of greater commercial success in industrialized countries will be considered. This requires definition of both the different ways baculoviruses can be used for insect control, and the performance expectations used to evaluate baculovirus success. Such an assessment identifies the key features required for a virus to be successful as a control agent. Though baculoviruses are used as the examples here, these principles apply to other pathogens, and many predators and parasites, as well.

The guiding principle in pest control remains primarily one of economics; control strategies and agents used are those that are the most cost-effective in the short or long term. This would appear to be obvious, but too often is overlooked in the literature on viruses and other biological control agents. The effectiveness of a control strategy based on a baculovirus will always be compared with that obtained with other available control strategies, especially those based on other pathogens and synthetic chemical insecticides. Cost-effectiveness can vary with such factors as crop, season, the species complex to be controlled, the level of control considered effective, the cost of production of the control agent, geographical location, governmental regulations affecting

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registration and use, and the status of economic development in the country where the virus is used. The latter two factors are particularly relevant, because the costs for development, production, and use of a baculovirus in developing countries are much less than in highly industrialized countries. This is a result of lower material and labor costs in developing countries, as well as to the smaller size of most farms, lower levels of agricultural mechanization, and cheaper and less cumbersome registration procedures. For these reasons, baculoviruses are used more in developing countries than in the highly industrialized nations, where they account for <0.1% of operational pest control. As a result, positive assessments of baculovirus cost-effectiveness, based on studies carried out in developing countries, cannot be directly translated into corresponding levels of cost-effectiveness in developed countries.

1.1. Strategies for Use

The most cost-effective potential strategy for using a baculovirus, as with other biological control agents, is as a classical biological control agent. In this strategy, introduction of a virus results in outbreaks of disease (epizootics) and maintenance of the virus in the target population. Within a few years, the pest population is reduced below the economic threshold on a permanent basis. Baculoviruses may establish and become endemic in a target population within a few years of introduction, yet there is only one good example of a classical biological control success with a baculovirus: the control the European spruce sawfly, *Gilpinia hercyniae*, in North America, by its nuclear polyhedrosis virus (NPV) (*see* **Subheading 3.1.**). Another strategy is to use a virus as an augmentative control agent. In this strategy, a virus endemic in a population, but at a low level, is applied against a pest population at the beginning of the season or early in the development of the pest population. The virus reduces the population below the economic threshold, or reduces pest damage substantially sooner than might occur naturally. The effect usually only lasts one or a few seasons, and must be repeated. The granulosis virus (GV) of the grapeleaf skeletonizer, *Harrisina brillians*, and the NPV of the Douglas fir tussock moth, *Orgyia pseudotsugata*, described in **Subheading 3.2.**, have been used successfully in this manner. Again, however, this tactic has proven of only limited utility.

The most common baculovirus pest control strategy is to use a virus as a viral insecticide. In this tactic, a virus, such as the *Heliothis* NPV, is formulated and applied against a target pest on a periodic basis, as needed, much as are chemical insecticides. Depending on the target pest and cropping system, applications may be fewer than those required with a chemical insecticide, because the viruses are quite specific and typically do not kill predatory and parasitic insects. Natural enemies, therefore, can remain in the ecosystem, retarding increases in the pest population after the initial mortality caused by the virus. In addition, viral reproduction in the target insect could add to the amount of the virus in the crop environment, and this can extend control and, thus, cost-effectiveness. In pests with only one or a few generations per season, a single application may yield effective season-long control, where a combination of these factors is in operation.

1.2. Performance Expectations and Economics

An issue of major importance affecting the adoption of baculoviruses as control agents is performance expectation. In most cases, a successful virus is one that can reduce the pest or vector population to below an economic threshold routinely and reliably, at a cost that is economical in proportion to the value of the crop. Clearly, viruses that are effective as classical biological control agents, or as augmentative agents, would be the most cost-effective, because of the limited number of applications required. But few viruses can be used successfully in either of these strategies. As a result, the efficacy of most viruses is evaluated in terms of their utility as viral insecticides.

Since World War II, the performance of chemical insecticides has set very high expectations for all alternative pest control strategies. Traditionally, chemicals have been fast-acting, broad-spectrum control agents with substantial residual activity, which are relatively inexpensive, and easy to produce, formulate, and use. Thus, under most circumstances, baculoviruses are evaluated on the basis of how they compare with chemical insecticides, in particular, how quickly they kill the target insect, and at what cost for an acceptable level of crop protection. This leads to a paradox for microbial control agents. The two properties of chemical insecticides originally considered their best attributes, i.e., a broad spectrum of activity and significant residual activity, are now viewed as detrimental, because they result in the destruction of natural enemy populations and the development of insecticide resistance, though the latter is often the result of overuse. Yet, most baculoviruses have a narrow spectrum of activity and relatively poor residual activity. Though these are now considered desirable properties, until recently, they have discouraged interest by industry in the development of many potentially useful viruses, because of the relatively high costs of development and registration in comparison to the likely return on investment.

The costs for development and registration of a naturally occurring baculovirus, however, are much cheaper than those for a chemical insecticide, e.g., approximately one-half million dollars for a baculovirus, compared to at least several millions of dollars for most chemicals in the United States, Europe, and Japan. But a company still must see the potential for making its investment pay off within a few years. For viruses highly specific in host range, unless their target is a pest of a major commodity, or a polyphagous pest causing damage to a variety of crops, this is simply not possible given the current regulatory environment and market size in most industrialized countries.

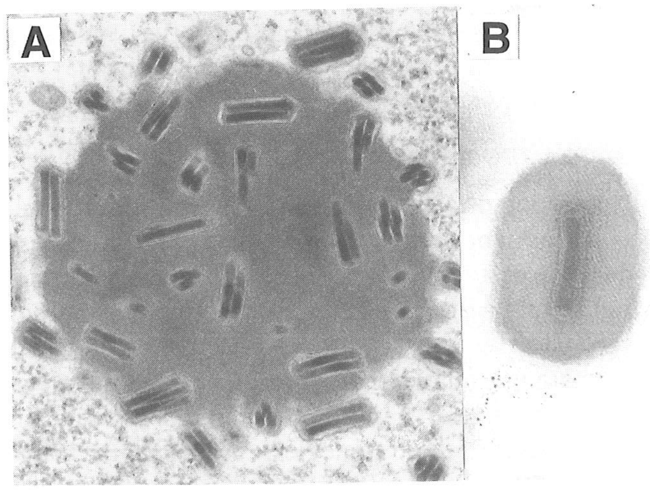

Fig. 1. Electron micrographs of **(A)** a polyhedron of a typical nuclear polyhedrosis virus, and **(B)** a granule of a granulosis virus. The NPV illustated is of the multinucleocapsid type (MNPV), with more than 1 nucleocapsid possible per viral envelope. Some NPVs have only a single nucleocapsid per envelope (SNPV type), but all GVs have only one nucleocapsid per envelope.

Industrial interest, whether on a small or large scale, is important, because it is industry that will produce the viral insecticides. This is particularly true in developed countries, where farms tend to be large. In fact, most farmers, whether large or small, want a reliable supply of control agents, and, though willing to change cultural practices, because of numerous other responsibilities, they typically are not willing to manufacture their own insecticides.

2. Baculovirus Types and General Properties

The baculoviruses compose a single family, Baculoviridae *(2)*, containing two genera of occluded viruses, *Nucleopolyhedrovirus*, commonly know as nuclear polyhedrosis viruses (NPVs), and *Granulovirus*, commonly known as granulosis virus (GV). The occluded viruses are so named because, after formation in infected cells, the mature virus particles (virions) are occluded within a protein matrix, forming paracrystalline bodies that are generically referred to as either inclusion or occlusion bodies (**Fig. 1**).

2.1. Biological Properties Related to Insect Control

A short description of the most important biological properties of NPVs and GVs is given here, to set the stage for discussion of how these viruses are used as insect control agents. Knowledge of these properties for different types of NPVs and GVs provides insight into the advantages and limitations of their use for insect control.

NPVs are known from a wide range of insect orders, as well as from crustacea (shrimp), but by far have been most commonly reported from lepidopterous insects, from which well over 500 isolates are known *(2)*. Many of these are different viruses (i.e., viral species), but, even in a general sense, it is not known how many viral species are represented among these isolates, because the baculovirus species concept is not well developed. NPVs are easily transmitted *per os* and replicate in the nuclei of cells, generally causing an acute fatal disease, with death usually occurring 5–9 d after infection in larvae infected during the third or fourth instar. The occlusion bodies of NPVs are referred to commonly as polyhedra, because they are typically polyhedral in shape (**Fig. 1A**). They are large (approx 0.5–2 μm), and form in the nuclei, where each occludes as many as several hundred virions. The NPVs of lepidopterous insects infect almost all host tissues, but produce the most polyhedra in the epidermis, fat body, and trachael matrix. Virion-containing polyhedra typically are not produced in the midgut epithelium. In other orders of insects, infection and production of polyhedra are typically restricted to the midgut epithelium. Some NPVs have a very narrow host range, and may only replicate efficiently in a single species; others, such as the AcNPV, i.e., the NPV of the alfalfa looper, *Autographa californica* (Speyer), have a relatively broad host range and are capable of infecting species from different genera *(2)*.

The GVs, of which there are now over 100 isolates, are closely related to the NPVs, but differ from the latter in several important respects. Like NPVs, GVs are highly infectious *per os*, but are only known from lepidopterous insects *(2)*. Initially, GVs replicate in the cell nucleus, but replication involves early lysis of the nucleus (prior to virion formation), which, in NPVs, does not occur until after most polyhedra have formed. After the nucleus lyses, GV replication continues throughout the cell, which consists of a mixture of cytoplasm and nucleoplasm. When completely assembled, the virions are occluded individually in small (200 × 600 nm) occlusion bodies, referred as granules (**Fig. 1B**). Many GVs primarily infect the fat body (type 1); others have a broader tissue tropism and replicate throughout the epidermis, tracheal matrix, and fat body (type 2). The type 1 and type 2 GVs, like the lepidopteran NPVs, do not produce occluded virions in the midgut epithelium *(3)*. One, however—the GV of the grapeleaf skeletonizer, *H. brillians*—is unusual because it only replicates in the midgut epithelium (type 3), where the occluded virions are produced *(3)*.

3. Use of Baculoviruses as Insect Control Agents

In this section, examples will be provided to illustrate the use of baculoviruses as classical biological control agents, augmentative control agents, and viral insecticides. Baculoviruses are most commonly used as viral insecticides, and, thus, this tactic will receive the most attention.

3.1. Classical Biological Control

The best example of insect control by a baculovirus is the use of the NPV of the European spruce sawfly, *G. hercyniae*, as a classical biological control agent to control this important forest pest in North America ***(4,5)***. The European spruce sawfly was introduced into eastern Canada from northern Europe around the turn of the century, and was a severe forest pest by the 1930s. Hymenopteran parasites were introduced from Europe in the mid-1930s as part of a biological control effort, and, inadvertently, along with these came the NPV, first detected in 1936. Natural epizootics caused by the virus began in 1938, by which time the sawfly had spread over 31,000 km^2. Most sawfly populations were reduced to below economic threshold levels by 1943, and remain under natural control today, the control being effected by a combination of the NPV, which accounts for more than 90% of the control, and the parasites. Although viruses, particularly NPVs, are frequently associated with rapid declines in the populations of important lepidopterous and hymenopterous (sawfly) pests, the *G. hercyniae* NPV is the only virus that proved effective as a classical biological control agent ***(5)***.

Because the *G. hercyniae* NPV remains the most successful example of pest control by a virus, it is worthwhile to consider why it has been so successful. Two main reasons are apparent: First, the virus attacks the midgut epithelium. As a result, within 48 h of infection, larvae begin to defecate newly produced infectious viral polyhedra. Each infected larva becomes a virus factory for distributing the virus in the environment. The second reason is that the virus can be passed vertically from adults to larvae. When an individual becomes infected in the last instar, it can often survive. The emerging adult then develops an infection in the midgut, and also disseminates the virus in the environment.

Another successful use of a virus as a quasiclassical biological control agent is the use of the so-called nonoccluded baculovirus of the palm rhinoceros beetle, *Oryctes rhinoceros,* (Scarabaeidae) for control of this scarab pest in the South Pacific. During the middle of this century, the palm rhinoceros beetle, an introduced pest, became a serious pest of oil and coconut palms in many South Pacific islands, including the Fiji Islands, Western Samoa, American Samoa, and the Tokelau Islands ***(6)***. The beetle adult bores into the heart of the palm, and, aside from damage to the fronds, repeated attacks can cause tree death. Eggs are laid, and larvae develop in decaying palm tissue, as well as in other types of decaying vegetable matter, and in manure. A search for pathogens led to the discovery of a nonoccluded baculovirus in Malaysia in the mid-1960s ***(7)***. This virus apparently did not occur in the islands, where the beetle had become a pest, and it was subsequently developed as a very effective control agent.

The virus develops in the midgut epithelium of larvae and adults, but also in other tissues, such as the fat body. Infection of the gut results in highly infectious feces, which in turn results in the virus being spread to other adults during the course of mating, and to larvae when infected females visit larval habitats for oviposition. Infection of females also reduces fecundity substantially. In the early stages of the control program, compost heaps and coconut logs were contaminated with virus, which resulted in both infected larvae and adults, with the latter serving to disseminate the virus to other natural habitats. When it was realized that the adults were susceptible to infection and could disseminate the virus very effectively, the control tactics focused on direct infection of adults ***(6)***. Wild adults were trapped, contaminated with virus by immersing them in aqueous virus suspensions (2–3 freshly ground virus-killed beetles/L) for several minutes, and then released to carry the virus into natural populations.

This program has been very successful in areas where the beetle was a major introduced pest. Damage to fronds in Fiji, for example, ranged from 40 to 90% prior to introduction of the virus. Within 4–6 yr in most of these areas, the level of beetle-damaged fronds was <20%, and, in many areas, <10% ***(6)***. Corresponding reductions in adult beetle catches also occurred. In areas where the concentrations of breeding sites were high, periodic beetle outbreaks can reoccur, necessitating reintroduction of the virus. With the exception of this requirement for periodic reintroductions of virus in some areas, the use of the *Oryctes* baculovirus could be considered a true classical biological control success.

3.2. Augmentative Control Agents

Baculoviruses have not been commercially successful as augmentative control agents, but two examples are worth mentioning: the use of a GV against the western grape leaf skeletonizer, and a NPV against the Douglas fir tussock moth. They illustrate how baculoviruses can be effective on a long-term basis, yet still do not attract industrial development.

The western grape leaf skeletonizer, *H. brillians*, can be a serious outbreak pest of table and wine grapes in many areas of California. In most cases, it can be controlled with applications of Kryolite or *Bacillus thuringiensis* (Bt). However, populations of *H. brillians* usually return in a generation or two, and must be treated again. Treatment of populations at a rate of 8–10 g/ha of GV-diseased cadavers, however, can yield mortality rates of greater than 90%, and reduction of the pest population to well below an economic threshold, for 3 yr or longer ***(8)***.

This excellent level of long-term control results from a unique combination of virus and host biology. Grape leaf skeletonizer larvae are gregarious, feeding side by side during the first three instars, and follow each other to new feeding sites as leaf tissue is consumed. The virus replicates in the midgut epithelium, and, within a few days of infection, a virus-laden diarrhea develops. Thus, once a single larva is infected, all the individuals within the population usually become infected and die within a week. In addition, larvae infected during the last instar survive and carry the virus over into the adult stage. Infected adult females generate fresh viral granules in their gut, and contaminate eggs with virus when the abdomen contracts during oviposition. So, like the sawfly and palm beetle viruses, infection of the midgut epithelium is the major factor contributing to the success of this virus, and this advantageous property is complemented by the gregarious behavior of the larvae *(8)*.

Ironically, as successful as this virus could be as a pest-control agent, it is not used commercially. The reasons are simple. The market is small, because the virus only has to be applied once or a few times every several years, making it difficult for industry to justify the registration costs. In addition, broad spectrum insecticides are available to control *H. brillians.* Methods for limited virus mass production are available that would enable commercial insectaries, which produce parasitic insects and predators, to produce the virus and make a profit. However, the costs for registration of the virus by the U.S. Environmental Protection Agency (EPA) and State of California are too high for these small-business insectaries to justify registration.

In the case of the Douglas fir tussock moth, *O. pseudotsugata*, the OpNPV that attacks this species can also be used very successfully to reduce damage to large areas of Douglas fir forests. The tussock moth is a cyclical problem, capable of causing significant losses every several years at the peak of its population cycle. As populations reach high densities, natural epizootics caused by NPV often reduce the population to well below an economic threshold for several years, but usually only after substantial economic damage has occurred. Thus, in the Douglas fir tussock moth control program, populations are monitored, and the virus is sprayed on populations to initiate epizootics before they reach an economic threshold. Tests in the field have shown that one application by aircraft of virus at a rate of 2.5×10^{11} polyhedra/ha reduces larval densities by 90%, and damage levels by 40–55%, with full recovery of foliage the following year *(9)*. A range of field trials in the 1970s made it clear the OpNPV can be used to successfully control the tussock moth. Yet, the periodic nature of the problem, and difficulties in mass production of the virus, have discouraged commercial development. Nevertheless, the OpNPV has been registered by the US Forest Service, and was produced periodically on a contract basis, most recently by Biosys of Columbia, MD. However, this firm went out of business in 1996.

3.3. Baculoviruses as Viral Insecticides

The viruses most commonly used or considered for development as microbial insecticides, in industrialized as well as less developed countries, are the NPVs (**Table 1**). GVs are used against pests for which no effective NPVs are known. NPVs have received the most study because they are common and easily isolated from many important lepidopteran pests, production in their hosts is straightforward and inexpensive, and the technology for formulation and application is relatively simple and adaptable to standard pesticide application methods. Typically, however, NPVs have a narrow host range, infecting only a few closely related species. Furthermore, though several can be grown in vitro in small volumes (ca. 80-L cell cultures), fermentation technology is not currently available for their mass production on a large-scale commercial basis. These two key limitations have proven significant disincentives for the commercial development of NPVs, especially in industrialized countries. The large chemical and pharmaceutical companies that might be expected to take an interest in NPVs, with rare exception, have shown little interest in producing a product with a small market, and which must be grown in live caterpillars or sawfly larvae. This type of technology is more suitable for cottage industries in industrialized or developing countries. But, in industrialized countries, where all pathogens used as insecticides must be registered by one or more governmental agencies, regulatory procedures and associated costs impede development by smaller companies, such as commercial predator and parasite insectaries.

Even with these drawbacks, several NPVs have been registered as microbial insecticides in industrialized countries (though few are currently marketed), and, whether registered or not, many are used in less-developed countries, particularly for control of lepidopteran pests of field and vegetable crops. Moreover, there is renewed interest in developing NPVs as insecticides, because of the adverse effects of chemical insecticides and their increasing costs, and because recombinant DNA technology offers potential for improving the efficacy of these viruses. For economic reasons, as in the past, this interest is still restricted largely to NPVs of major lepidopteran pests and a few key sawfly pests. The high host specificity of NPVs makes them of most use where a single insect species is the only pest, or at least a key one of a particular crop. Utility is increased if the target insect is not very sensitive to Bt, and is resistant to chemicals, or the latter are too costly, environmentally unacceptable, or unavailable. The viruses listed in **Table 1** all meet, or at one time met, these criteria. The possibilities for improvement through genetic engineering are dis-

Table 1
Major Baculoviruses Registered for Control of Insects Pests

Target pest	Virus	Crop	Product name	Producer	Country
Caterpillars					
Anticarsia gemmatalis	AgNPV	Soybeans	VPN	Agricola El Sol	Brazil
Adoxophyses orana	AoGV	Fruit orchards	Capex	Andermatt Biocontrol	Switzerland
Cydia pomonella	CpGV	Apples, walnuts	Madex	Andermatt Biocontrol	Switzerland
			Granupom	AgrEvo	Europe
			Carpovirusine	NPP (Calliope)	France
Helicoverpa zea	HzNPV	Cotton, vegetables	Gemstar	Thermo Trilogy	Columbia, MD
Heliothis virescens					
Lymantria dispar	LdNPV	Deciduous forests	Gypcheck	Thermo Trilogy	Columbia, MD
			Disparvirus	Canadian Forest Service	Canada
Mamestra brassicae	MbNPV	Vegetables	Mamestrin	NPP (Calliope)	France
Orgyia psuedotsugata	OpNPV	Douglas fir forests	TM Biocontrol-1	Thermo Trilogy	Columbia, MD
Spodoptera exigua	SeNPV	Vegetables, flowers	Spodex	Thermo Triology	Columbia, MD
Spodoptera littoralis	SlNPV	Cotton	Spodopterin	NPP (Calliope)	France
Sawfly larvae					
Neodiprion sertifer	NsNPV	Pine forests	Neocheck-S	Canadian Forest Service	Canada
			Sentifervirus		
Neodiprion lecontei	NlNPV	Pine forests	Lecontivirus	Canadian Forest Service	Canada

cussed in Chapter 15; here, the focus is on naturally occurring baculoviruses as viral insecticides.

3.3.1. Production In Vivo and Formulation

Baculoviruses are mass produced in larval hosts grown on an artificial diet or natural host plants. To maximize production of NPVs, larvae are infected *per os* at an advanced stage of development, such as during the late fourth instar, and reared either in groups, or individually, for species that are cannibalistic. After ingesting the virus, the occlusion bodies dissolve in the alkaline midgut, releasing the virions. In lepidopterans, the virus first invades midgut epithelial cells, where, during the first 24 h of infection, it undergoes an initial colonizing phase of replication in the nuclei of these cells. No occlusion bodies are produced in these nuclei, but rather the progeny virions migrate to the tracheal matrix or through the basement membrane, and invade and colonize almost all other tissues of the host. In these, the occlusion phase of replication occurs, during which the virions are occluded in polyhedra. Maximum production of polyhedra occurs in tissues that are the most nutrient rich and metabolically active, such as the fat body, epidermis, and tracheal matrix ***(3)***. The occlusion phase of viral disease occurs over a period of 5–10 d, and represents several cycles of replication as the virus spreads throughout the tissues and invades most host cells. The actual length of the disease depends on several factors, including the host and viral species, larval instar at the time of infection, amount of inoculum, and temperature. Near the end of the disease, after most polyhedra have formed, the nuclei lyse. As more and more nuclei lyse, the larva eventually dies, after which the body liquefies, releasing literally billions of polyhedra. In commercial production, larvae may be harvested prior to liquefaction, to keep bacteria, which quickly colonize dead larvae, at a low level in the final product. Alternatively, antibiotics can be added to the diet to keep bacterial counts low. After the larval production phase is complete, the larvae are collected and formulated.

Formulation varies, depending on how the virus will be used ***(10–12)***. Both liquid and dust formations of viruses have been developed and tested, with and without additives, such as UV light protectants, antioxidants, gustatory stimulants, and spreader-stickers. Additives often show improvements in efficacy in laboratory tests, and sometimes in percent larval mortality in field trials, but not usually when the criterion is crop yield. Thus, their additional cost generally reduces cost-effectiveness. Recently, however, the addition of stilbene brighteners has been shown to increase the efficacy of the NPV of the gypsy moth, *Lymantria dispar* (LdNPV; ***13***). In addition, the inclusion of molasses (5–25%) in liquid formulations has been shown to improve the efficacy in the field of some NPVs, such as the Op and LdNPVs ***(9,14)***.

The production of lepidopteran GVs and sawfly NPVs is similar to that described for lepidopteran NPVs ***(5,11)***. As noted, however, the sawfly NPVs differ from the lepidopteran NPVs, in that the former only replicate and form polyhedra in midgut epithelial cells. Polyhedral yields, therefore, are lower than those obtained with lepidopteran NPVs, but field application rates are correspondingly lower ***(5)***.

3.3.2. Efficacy and Use of Baculovirus Insecticides

The extent to which baculoviruses can be useful as insecticides depends on several factors, including the relative importance of the target pest in the pest complex attacking a crop, the amount of virus that must be used to control the pest in both the short term and long term (persistence and carryover), the value of the crop, and the cost and availability of alternative control measures. Baculoviruses are good candidates for use where a single lepidopteran species is the major pest for most of the growing season on a crop with a high cash value, when other available pest control methods are not cost-effective. Examples include NPVs effective against insecticide-resistant species of *Helicoverpa*, *Heliothis*, and *Spodoptera* on such crops as cotton, corn, and sorghum, and, even more so, on tomatoes, strawberries, and flowers such as chrysanthemums. The cost-effectiveness of these viruses is determined by the amount of virus that must be applied, and the frequency of application necessary to keep the pest below an economic threshold. As noted earlier, this will vary with the virus, pest, crop, and, more importantly, among different countries. The amount of virus required to achieve effective control is typically expressed in terms of the number of polyhedra (for NPVs) or granules (for GVs) that must be applied per unit area, e.g., acres or hectares. To correlate this with larval production costs, the number of application rates for different viruses can be translated into the number of larval equivalents (LEs)* required per hectare; illustrative examples are given in **Table 2**.

The number of LEs required to obtain effective control is critical to determination of cost-effectiveness, because of the cost of labor and materials that go into virus production. The best results with baculoviruses are obtained against moderate infestations of early- to mid-instar larvae. Treatment of heavy

*A value often used for the larval equivalent is 6×10^9 polyhedra/larva. This value is based on the number of polyhedra typically obtained from a fifth-instar larvae of *H. zea* ***(10)***. However, the actual number of polyhedra obtained from a larva varies considerably with the virus isolate and species of insect used for production. Therefore, the number of larval equivalents required for effective control is best determined empirically through field trials, and can then be expressed in terms as LEs, as well as the number of polyhedra required per treatment for effective control. Regardless of the methods for quantifying the virus, its biological activity must also be established through bioassays.

Table 2
Occlusion Body Yields and Typical Application Rates for Representative Baculoviruses

Virus	Occlusion body Yield/larva	Occlusion body Application rate/ha	Larval equivalents/ha[a]
Helicoverpa zea NPV	6×10^9	2×10^{11}	33
Lymantria dispar NPV	2×10^9	5×10^{11}	250
Orgyia pseudotsugata NPV	2×10^9	3×10^{11}	125
Spodoptera exigua NPV	2×10^9	1×10^{12}	500
Neodiprion sertifer NPV	3×10^7	7×10^9	200
Cydia pomonella GV	1×10^{11}	2×10^{13}	200

[a]Amount required to a reduce crop loss or pest population below economic threshold.

infestations of advanced instars (i.e., late fourth-instars and beyond) is typically not effective. Rates against moderate infestations can range from 150 LEs/ha/treatment, using the *H. zea* NPV to control *Heliothis virescens* on cotton, to 500 LEs for the *Spodoptera exigua* NPV on lettuce. The number of LEs required to control a specific pest can vary with the crop, because of differences in plant phenology and chemistry. This type of information is essential for evaluating whether a specific NPV merits commercial development, as well as use against a specific insect on a particular crop.

There have been many reviews over the years on the use of viruses as control agents ***(14,16–22)***. Rather than re-examine this information again in detail, relevant data for key baculoviruses registered in the United States will be used here to illustrate the above economic concepts and rates of application that are considered to be effective.

3.3.2.1. *Heliothis* NPV

Larvae of the noctuid moths, *H. zea*, *Heliothis armigera*, and *H. virescens*, continue to be major pests of crops, such as cotton, corn, tobacco, soybeans, and tomatoes, in the United States and many other countries. These species are susceptible to a virus known as the *H. zea* NPV (HzNPV). Because of the economic importance of the crops attacked and damage inflicted by these pests, especially *H. virescens* on cotton, the HzNPV was the first virus developed commercially in the United States ***(10)***, and is one in which there remains considerable interest. The HzNPV was originally marketed under the trade name Elcar by Sandoz (Basel, Switzerland). However, it came to market at about the same time as the pyrethroid insecticides, and the lower cost and higher efficacy of the latter essentially eliminated the Elcar cotton market. Over the past decade, high levels of resistance to pyrethroid insecticides has renewed interest in the HzNPV, and Thermo Trilogy in the United States (Columbia, MD) is currently producing the virus under the product name Gemstar.

The best results with the HzNPV have been obtained on cotton, where, in the United States, the major pests are *H. virescens* and *H. zea*. Treatment of low to moderate infestations at a rate of from 10×10^{10} polyhedra/0.4 ha, and 45×10^{10} polyhedra/0.4 ha for heavy infestations, resulted in yields comparable to those obtained with chemical insecticides ***(15)***. Results obtained on other crops showed the virus was not as effective against these noctuid pests as it was on cotton ***(15)***.

3.3.2.2. *Spodoptera exigua* NPV

The beet armyworm, *S. exigua*, is a polyphagous insect that has emerged as one of the most important pests of vegetable crops in the world. It is a particularly important pest of tomatoes, celery, alfalfa, and strawberries in the United States, a major vegetable crop pest is Southeast Asia, as well as a pest of chrysanthemums in the United States and The Netherlands. Much of its pest status is attributable to its resistance to most chemical insecticides. The high cash value of many crops attacked by the beet armyworm led to the development of the *S. exigua* NPV (SeNPV) as the viral insecticide, Spodex. Spodex is sold in The Netherlands for use against the beet armyworm on chrysanthemums, and in Southeast Asia for use on vegetable crops. Registration is also being sought in the United States for use on vegetable crops, especially fresh market tomatoes.

Field trials of the SeNPV have shown that it is most effective when used at rates of from 2 to 4×10^{12} polyhedra/ha, whether applied in the field on lettuce, or in glasshouses on chrysanthemums ***(16)***. Use of Spodex against forage crops, such as alfalfa, is generally not cost-effective, because of the low value of the crop/ha.

3.3.2.3. *Lymantria dispar* NPV

The gypsy moth, *L. dispar*, is an important pest, periodically, of deciduous trees in the northern hemisphere. Populations are cyclical, but, at their peaks, larvae are capable of total defoliation of hundreds of square kilometers of forests in many areas of the eastern United States, Canada, eastern Europe, and Russia. The US Forest Service developed and registered a product known as Gypcheck, which contains as its active ingredient the *L. dispar* NPV (LdNPV), a virus that is highly specific for gypsy moth larvae. Larvae can be controlled with chemical insecticides, Bt, and the LdNPV, but, in environmentally sensitive areas, such as most of the deciduous forest in the eastern United States, the virus is considered by many a better choice, because of its selectivity.

Field trials with Gypcheck have shown that it gives acceptable levels of tree protection when sprayed twice at a rate of 10^{11} polyhedra/0.4 ha ***(14)***. Acceptable control is considered a reduction of 50% or more in defoliation, which would have occurred in the absence of treatment.

3.3.2.4. *Neodiprion sertifer* NPV

The European pine sawfly, *Neodiprion sertifer*, is a major pest of pine forests in the United States, Canada, and Europe. Because of its importance, the *N. sertifer* NPV (NsNPV) is the sawfly virus that has received the most attention as a control agent. It has been registered for use against the European pine sawfly in the United States and Canada by their respective forest service agencies.

Numerous field trials conducted with the NsNPV have shown that very good control can be obtained when the virus is applied at a rate of $5–9 \times 10^{9}$ polyhedra/0.4 ha against moderate infestations of early-instar larvae ***(5)***. Higher rates (10^{10}) were required for heavier infestations ***(5)***.

3.3.2.5. *Cydia pomonella* GV

One of the most important insect pests of apples worldwide is the codling moth, *Cydia pomonella*. This species also attacks other tree crops, including plums, pears, and walnuts. On apples, larvae burrow directly into the fruit right after hatching. Thus, they represent a difficult target for a microbial pesticide because of the short period of their exposure. No NPVs effective against the codling moth are known, so the only virus developed to date for control of this important pest is the *C. pomonella* GV (CpGV).

The CpGV has been registered for use in both the United States and Switzerland. The apple growing conditions in these two countries are very different. In Switzerland, the cooler climate results in lower codling moth populations and a shorter pest season. In the United States, especially in northern California, the warmer climate at lower elevations results in larger moth populations and a pest season that can last for 10 wk. Thus, the total amount of virus that must be applied to get a marketable fresh apple crop in California is much higher than it is in Switzerland. For this reason, the CpGV has found a steady market in Switzerland, but has not found a commercial producer in the United States. However, apple farm cooperatives, especially those that grow organically grown apples (no synthetic chemical pesticides or fertilizers), are currently developing plans to produce the CpGV.

Regarding rates of application, control is effective when the virus is sprayed at a rate of 10^{13} granules/0.4 ha. In California, effective control requires one treatment per week over a 10-wk period. In Switzerland and other cooler climates, three treatments per season will usually yield effective control of codling moth larvae.

3.3.2.6. *Autographa californica* NPV

The viruses discussed above all have a narrow host spectrum, typically only infecting the target species and a few other closely related species. From an economic standpoint, this is often considered a disadvantage of viral insecticides. Most of the larger companies interested in viral insecticides would consider a virus that had a broad host range, but one restricted to lepidopterans, to be a good candidate for development. For this reason, the NPV of *A. californica* NPV (AcNPV), which is capable of infecting more than 50 lepidopteran species, has received considerable attention as a viral insecticide, and was recently registered by Biosys as Gusano. Because Biosys went out of business in 1996, it is not clear whether the naturally occurring version of this virus will be developed further and marketed. However, the AcMNPV is the virus that most groups in academia and industry have used as a model for improving insecticidal efficacy through genetic engineering (*see* Chapter 15).

3.3.2.7. *Anticarsia gemmatalis* NPV

The velvetbean caterpillar, *Anticarsia gemmatalis*, is a major pest of soybeans in many soybean growing areas, but particularly in Brazil. More than 15 yr ago, the Brazilian government mounted a control program aimed at developing the *A. gemmatalis* NPV (AgNPV) as a viral insecticide. Though this virus has not been registered in the United States, it is worthy of mention, because it has been one of the most successful documented control programs using a virus as an insecticide. The AgNPV is now used to control the velvetbean caterpillar on approx 1 million ha of soybeans in Brazil. The virus is produced both by inoculation of larvae in production colonies, and by field collection of larvae that have died from NPV disease ***(23,24)***. Rates of application vary with the infestation, but generally are in the range of 1–2 × 10^{12} polyhedra/ha.

3.4. Use of Baculoviruses in Developing Countries

Baculoviruses are used in many developing countries to control lepidopteran pests on field and vegetable crops, and even, in some cases, to control forest pests. Examples include the use of various *Spodoptera* NPVs to control *S. littoralis* and *S. litura* of field and vegetable crops in India, Africa, China, and other countries in southeast Asia; the use of the *Spodoptera frugiperda* NPV to control *S. frugiperda* on corn in Latin America; the HzNPV to control the *H. armigera* on cotton in southeast Asia, and *Heliothis/Helicoverpa* on cotton and vegetables in Latin America and India, and the use of the *Pieris* GV to control cabbage worms in China. In most cases, the use of these viruses is much more extensive than in industrialized countries. Thus, on a local basis, these viruses are successful control agents. But, as noted above, these examples

cannot be used to conclude that baculoviruses can be used successfully in developed countries, because the economic conditions are so different. In addition, the systems of evaluation differ. For example, cost-effectiveness has typically not been evaluated in a country like China. Methods have been developed for virus production, and the virus is simply sprayed on the crop, frequently or infrequently, to eliminate the pest. Communes and farms responsible for production of vegetable and field crops have their own virus production facilities, and ample labor, so the calculation of labor costs attributable to virus production, and corresponding levels of control, are not determined. This will probably change as the economy in China continues to expand and cost effectiveness is more carefully scrutinized. However, even in Latin American countries, the NPVs are considered successful in vegetable crop production. The reason is that labor costs are relatively low, and virus can be produced cheaply in small insectaries, or by collection of virus-infected larvae in the field at the end of the season. Baculoviruses have been used in developing countries for decades, and this use would probably not have continued if the viruses were not considered cost-effective under local economic conditions.

4. Conclusions

Viruses are not widely used at present in industrialized countries, because chemical insecticides are still readily available and effective, and because viruses have what are considered key limitations. In comparison to chemical insecticides, these limitations include a relatively slow speed of kill, a narrow spectrum of activity, little residual activity, and lack of a cost-effective system for mass production in vitro. On the other hand, these limitations have not inhibited the development and use of baculoviruses in several niche markets in the United States and Europe. In addition, NPVs and GVs are used in developing countries, especially on field and vegetable crops in China, India, and Brazil, as well as in many smaller countries in Latin America, Africa, and southeast Asia. The latter use results from the high cost of chemical insecticides in many of these countries, the development of insecticide resistance, low-to-moderate labor costs for virus production in vivo, and from the fact that registration for use of viruses is either not required or is easily obtained.

As pressure mounts to reduce the use of synthetic chemical insecticides, viruses may receive increased attention as alternatives to chemicals, particularly for the control of lepidopterous insects, for which no other effective control agents, such as Bt and parasites, exist. This may lead to an increased effort to develop and use conventional viruses in IPM programs in both industrialized and developing countries. In addition to conventional viruses, the development of recombinant DNA technology, i.e., genetic engineering, offers considerable promise for improving viral efficacy by reducing or eliminating the major disadvantages of viruses (*see* Chapter 15).

Some hope for the use of viruses in industrialized countries comes from the relative success of the bacterium Bt. Bt has been successful, even though it is only 2% of the insecticide market, because it either did not have the major limitation of viruses, or it overcame them. For example, Bt's performance compares favorably with chemical insecticides in many crop and forest systems in which lepidopterous insects are key or major pests. It is fast-acting, relatively inexpensive, and easy to produce, formulate, and use. It has a relatively narrow spectrum of activity, and its residual activity is rather low. Yet the range of insects Bt controls continues to provide a market large enough to justify commercial development. The potential for baculoviruses exists largely for pests in which Bt products are not effective, or in which resistance against these products or Bt transgenic plants is anticipated. Still, the commercial success of viruses on a large scale, for crops such as cotton and corn, will require the development of techniques for mass production in vitro to meet market demand, and viruses that act more quickly to prevent feeding damage than naturally occurring viruses. On a smaller scale, there are many NPVs that could be used in industrialized countries for existing and new niche markets.

Last, it should be noted that the window of opportunity for viral insecticides could be narrowing in industrialized countries because of several technological developments. These include the development new types of narrow-spectrum synthetic chemical insecticides, better strains of Bt targeted against *H. zea* and *H. virescens* in the cotton market (the largest potential market for viral insecticides), and the development of insecticidal transgenic cotton and corn based on Bt proteins, again targeted against the same above pests as are the viral insecticides. The development of significant resistance to any of these new technologies will continue to provide opportunities for viral insecticides, but this window, for both naturally occurring and recombinant baculoviruses, could well be limited to the next decade.

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17

Recombinant Baculoviruses

Michael F. Treacy

1. Introduction

Baculoviridae is a family of occluded, invertebrate-specific pathogens, consisting of two genera: the nucleopolyhedroviruses (NPVs) and granuloviruses. The majority of basic and applied research efforts, as well as commercial endeavors, have been focused on NPVs. In addition to vertebrate–invertebrate selectivity, many NPVs are infectious against only certain species within the insect order Lepidoptera, and impart no direct adverse effects on members of other insect orders, such as Coleoptera, Hymenoptera, Neuroptera, and Diptera *(1)*. Examples of lepidopteran-specific baculoviruses are those isolated from gypsy moth, *Lymantria dispar* (LdNPV), celery looper, *Anagrapha falcifera* Kirby (AfNPV), beet armyworm, *Spodoptera exigua* (Hubner) (SeNPV), and cotton bollworm, *Helicoverpa zea* (Boddie) (HzNPV).

Target specificity of NPVs make them good candidates for use in integrated pest management systems. Although several baculoviruses have been registered as commercial products, they have not gained widespread use in intensive agronomic systems. Two properties of NPVs that limit their utility as foliar insecticides are photolability and the requirement for ingestion by the pest, both of which delay or reduce acquisition of a lethal dose by targeted insects. Another important limitation of NPVs, and the focus of discussion in this chapter, is the length of time required to kill an infected insect. Depending on the virus and pest species, it may take nearly a week or longer before an infected insect dies. Further, an NPV-infected larva continues to feed until time of death. Instead of providing rapid curative action, as is commonly achieved with a synthetic insecticide, a foliar application of an NPV may allow too much crop damage to occur before the pest population is brought under control.

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