

A brief review of the past use of baculoviruses for the management of eruptive forest defoliators and recent developments on a sawfly virus in Canada

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ABSTRACT

An overview is presented of the state of knowledge on the use of baculoviruses—a family of virulent insect viruses exhibiting narrow host ranges—for the suppression of insect outbreaks in Canadian forests. Emphasis is on recent investigations using NeabNPV against the balsam fir sawfly and the pine false webworm.

Key words: *Acantholyda erythrocephala*, Baculoviridae, Baculovirus, balsam fir sawfly, biological control, forest protection, insect outbreak, *Neodiprion abietis*, pine false webworm

RÉSUMÉ

Une vue d'ensemble de l'état actuel des connaissances sur l'utilisation des baculovirus pour la suppression d'épidémies d'insectes dans les forêts du Canada est présentée. Les baculovirus sont membres d'une famille de virus d'insectes hautement virulents et possédant une spécificité d'hôte élevée. L'emphasis est placée sur des investigations récentes utilisant NeabNPV contre le diprion du sapin baumier et le pamphile du pin.

Mots clés : *Acantholyda erythrocephala*, Baculoviridae, Baculovirus, contrôle biologique, diprion du sapin, épidémie d'insectes, pamphile du pin, protection des forêts, *Neodiprion abietis*



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Introduction

This paper examines the use of baculoviruses (Baculoviridae) for the management of eruptive defoliators in Canadian forests. Significant knowledge has been gained since the last reviews on the subject (Cunningham and Kaupp 1995, Wallace and Cunningham 1995) to justify an update on the state and direction of research in this field. First, a general description of baculoviruses, their role in the ecology of eruptive forest insects, and their potential in biological control will be presented. Then, past use of baculoviruses in the forest will be summarized up to and including the most recent reviews and product registration (1892–1997). Finally, we will present an overview of current research in insect pest management

using baculoviruses in Canadian forests, and conclude with some lessons from past experiences, and suggestions for future research.

Baculoviruses and Eruptive Forest Insects

The cyclical population fluctuations of several species of forest insects have been associated with pathogens, particularly baculoviruses (e.g., Campbell 1963, Tanada 1976, Olofsson 1987, Myers 2000, Moreau 2004). The Baculoviridae constitute a family of viruses with covalently closed, double-stranded DNA genomes that range in size from 82 to 180 kilobases (Herniou *et al.* 2004, Lauzon *et al.* 2004). The rod-shaped virions, occluded in a proteinaceous occlusion body (OB) (Fig. 1) that can persist outside the host for a considerable period of time (Clark 1956, Thompson *et al.* 1981) must be ingested or vertically transmitted to cause infection (Cory and Myers 2003). Baculoviruses have been reported exclusively from arthropods, and have been recovered and genome sequenced so far only from Diptera, Hymenoptera, and Lepidoptera (Herniou *et al.* 2004). Based on OB morphology, two distinct genera have been defined within baculoviruses: *Granulovirus* (GV) and *Nucleopolyhedrovirus* (NPV). Recent analyses of fully sequenced baculovirus genomes have suggested that the NPVs of Diptera (Afonso *et al.* 2001) and Hymenoptera (Garcia-Maruniak *et al.* 2004, Lauzon *et al.* 2004, Duffy *et al.* 2006) are ancestral to lepidopteran NPVs and that the dipteran, hymenopteran, and lepidopteran NPV lineages diverged prior to the separation of GVs and NPVs

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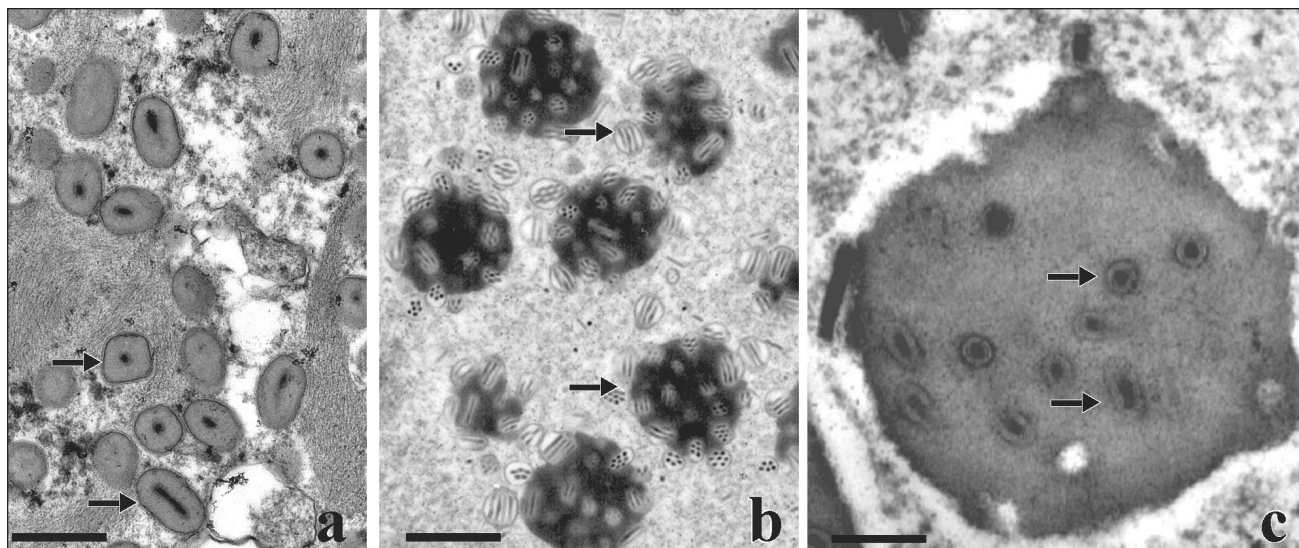


Fig. 1. (a) Spruce budworm (*Choristoneura fumiferana*) granulovirus (CfGV). Single virions (arrows) are enveloped and occluded in granulin protein. (b) Oblique-banded leafroller (*Choristoneura rosaceana*) nucleopolyhedrovirus (CrMNPV). Several virions are enveloped together (arrows) and then many enveloped groups of virions are occluded in polyhedrin protein. (c) Balsam fir sawfly (*Neodiprion abietis*) nucleopolyhedrovirus (NeabNPV). Several singly enveloped virions (arrows) are occluded in polyhedrin protein. (Scale bars: (a) 1 μ m, (b) 5 μ m, (c) 0.1 μ m).

within the Lepidoptera (Lauzon *et al.* 2004). Thus, GVs have only been reported from Lepidoptera, where they replicate in cells of the larval fat body, trachea, and epidermis (Federici 1997). A notable exception is the GV of the grapeleaf skeletonizer (*Harrisina brillians* Barnes and McDunnough), which replicates only in the cells of the midgut epithelium (Federici and Stern 1990). Likewise, hymenopteran NPVs are restricted to the midgut epithelium, primarily in the larval stages (Federici 1997), and dipteran NPVs infect only midgut epithelial cells in the gastric caeca and posterior stomach (Afonso *et al.* 2001). Initially, NPVs infect larval midgut cells in the Lepidoptera. However, the infection soon spreads to cells and tissues inside the hemocoel such as hemocytes, trachea, and fat body (Federici 1997). By the time this has occurred, the gut is in the process of curing itself by sloughing and replacing infected cells with healthy ones. When NPV-infected lepidopteran larvae finally die, they consist of little more than their exoskeletons restraining massive amounts (10^9 to 10^{10}) of viral OBs (Volkman 1997).

The role of baculoviruses in the population dynamics of their respective hosts is extremely variable from species to species. In some species, such as the spruce budworm (*Choristoneura fumiferana* Clemens), naturally occurring baculovirus epizootics have never been observed in the field to terminate an outbreak (Cunningham and Kaupp 1995, Lucarotti *et al.* 2004). In contrast, naturally occurring epizootics of baculoviruses are invariably associated with population declines in some other species, such as Douglas-fir tussock moth (*Orgyia pseudotsugata* McDunnough) and several species of sawflies (Hymenoptera, suborder Symphyta) (Coppel and Mertins 1977, Shepherd and Otvos 1986, Moreau 2004). The factors that can account for differences in baculovirus impact on host populations are (i) the degree of baculovirus infectivity and virulence, (ii) the host tissues affected by the baculovirus, and (iii) the probability of contact between the baculovirus and feeding larvae in nature

(Orlovskaya 1998). To this list, it may be appropriate to add a fourth factor, namely the habitat of the insect, as the success of a viral parasite is intricately associated with the larval habitat. These same criteria can be used to determine the potential, and strategy for use, of baculoviruses in insect pest control programs (i.e., classical biological control, augmentation of natural antagonist, or inundative augmentation).

The application of baculoviruses to forest ecosystems can generally be described as an augmentation of an indigenous pathogen reservoir rather than a classical introduction. To maximize the possibility of horizontal transmission within and between populations, such applications are ideally conducted shortly after egg hatch. Baculovirus effects may extend beyond the year of application, contributing to population suppression over several generations (Otvos *et al.* 1987, Moreau *et al.* 2005). Among the mechanisms contributing to carry-over effects between generations are OB persistence in the environment, transovum and transovarial transmissions (Cory and Myers 2003), and the presence of infectious cadavers on the foliage in the years that follow epizootics (Moreau *et al.* 2005). Additionally, both vertebrate and invertebrate natural enemies of the host can disseminate OBs outside treated areas, triggering epizootics in satellite host populations (Entwistle *et al.* 1983, Boucias *et al.* 1987, Vasconcelos *et al.* 1996).

Baculoviruses represent a promising alternative to the use of synthetic chemical insecticides because they often exhibit high virulence, have relatively narrow or specific host ranges, and have virtually no vertebrate or environmental toxicity and can therefore be applied aerially (Hunter-Fujita *et al.* 1998). Baculoviruses, especially NPVs, have been the subject of extensive safety testing in accordance with many of the same specifications that have been applied to chemical pesticides. The extensive scientific literature on the health and environmental safety of baculoviruses has been well reviewed (see reviews by Ignoffo 1975, Cunningham and Entwistle 1981, Gröner 1986, Anon. 2002). These baculoviruses have

been ingested and inhaled by, injected (intravenous, intraperitoneal, intramuscular) into, and applied to the skin and eyes of test animals without detrimental effects that could be attributed to the baculovirus tested. Also, no evidence for baculovirus-induced cytogenic, carcinogenic, mutogenic or teratogenic effects in vertebrate cells has been found (Ignoffo 1975, Gröner 1986, Anon. 2002). However, baculovirus multifactorial speed of action, instability in the field, high production cost, and short shelf life compared with chemical insecticides (but see Otvos *et al.* 2006) have often limited their use for insect pest management (Leisy and Fuxa 1996, Moscardi 1999, Copping and Menn 2000, Inceoglu *et al.* 2001). Nevertheless, in contrast with other biological control agents, the history of baculoviruses used as control agents is devoid of any documented observations of unexpected or undesired repercussions (Ehler 1991).

Baculoviruses as Biocontrol Agents of Eruptive Forest Defoliators: 1892–1997

The earliest attempt to control an eruptive forest defoliator using a baculovirus can be dated to 1892, when an NPV was applied (i.e., augmentation) against natural populations of its host, the Nun moth (*Lymantria monacha* L.), in Europe (Huber 1986). But, due to inappropriate methods of formulation preparation, the observed collapse of populations following applications was probably caused by naturally occurring epizootics rather than the augmentation efforts (Huber 1986). Ironically, the first attempt to use a baculovirus in North American forests was directed against accidentally released individuals of the same genus, the gypsy moth (*Lymantria dispar* L.), with the introduction of LdMNPV in 1913 in the United States in an operation that met with moderate success (Cunningham 1995). The first successful case of controlling an eruptive forest defoliator using a baculovirus occurred in Canada during the 1930s. In that period, the European spruce sawfly (*Gilpinia hercyniae* Hartig), an exotic Eurasian species, had become a major defoliator of spruce in both Canada and the United States. Fortunately, the importation and release of natural parasitoids from Scandinavia also accidentally introduced an NPV that quickly spread within and between populations, triggering a series of large-scale epizootics (Bird and Elgee 1957). By 1945 the European spruce sawfly outbreak in eastern Canada was over and since then, no control measures have been required due to the action of the virus and parasitoids (Neilson and Morris 1964).

In the decades that followed, numerous investigations were undertaken to examine the potential of different baculoviruses for the control of eruptive forest insects using different methods (see two earlier reviews in Cunningham and Kaupp 1995, Wallace and Cunningham 1995). In these

investigations, baculovirus OBs were mass produced either by infecting larvae in laboratory rearing or by harvesting dead larvae from field populations that had been previously treated at high rates with the virus. After OB purification from dead larvae or grinding of freeze-dried cadavers to fine powder, OBs were generally suspended in a water solution containing molasses, sometimes sticker products, and rarely, optical brighteners (reviewed in Cunningham and Kaupp 1995, Wallace and Cunningham 1995). Applications were made in ground spray trials, and also in aerial spray trials using helicopters or small fixed-wing airplanes. Although effective population suppression was obtained in some of the more than 20 different baculovirus/host systems investigated (Cunningham and Kaupp 1995, Wallace and Cunningham 1995), only four baculoviruses have been successfully developed and registered for use in Canadian forests in this period (Table 1). Among the factors that have limited baculovirus registration are the costs associated with production, and the absence of a sustained regular/continuous demand for registered products. Consequently, the use of baculoviruses to suppress eruptive forest insects has remained relatively marginal compared with other control measures such as *Bacillus thuringiensis* Berliner (Bt) (Lacey *et al.* 2001).

Baculoviruses as Biocontrol Agents of Eruptive Forest Defoliators: 1997–2005

Balsam fir sawfly

The balsam fir sawfly (*Neodiprion abietis* Harris) is a defoliator of firs (*Abies* spp.) and spruces (*Picea* spp.), and is widely distributed throughout North America (Wallace and Cunningham 1995). Historically, the strategy used against eruptive populations of the balsam fir sawfly was to wait for the natural collapse of populations, as outbreaks of this defoliator were not known to last more than 3 or 4 years and were not likely to spread rapidly (Smith 1947). Therefore, suppression measures were not considered when an infestation of this insect developed in 1990 in Bottom Brook, on the west coast of the island of Newfoundland. This infestation, although severe, remained relatively localized in its initial 5 to 6 years (Moreau 2006). However, when it became apparent in 1996–1997 that this outbreak was not collapsing as previous outbreaks had, an extensive research program was launched by the Canadian Forest Service, the University of New Brunswick, and several industrial, academic, and governmental partners to examine balsam fir sawfly ecology, distribution, and impact on the forest resource, and to identify potential biocontrol strategies (Lucarotti *et al.* 2007). This outbreak eventually developed into what is now the longest and most severe outbreak of this insect in western Newfoundland, and probably across Canada (Moreau 2006).

Table 1. Baculoviruses registered in Canada as of 1 July 2006.

Year of registration	Trade name	Scientific name	Registered against
1983	Lecontvirus WP	NeleNPV	<i>Neodiprion lecontei</i> (Fitch)
1983	Virtuss®	OpMNPV	<i>Orgyia pseudotsugata</i> (McDunnough)
1987	TM Biocontrol-1	OpMNPV	<i>Orgyia pseudotsugata</i> (McDunnough)
1997	Disparvirus	LdMNPV	<i>Lymantria dispar</i> (L.)
2006	Abietiv™	NeabNPV	<i>Neodiprion abietis</i> (Harris)

Table 2. Aerial applications of NeabNPV against the balsam fir sawfly in western Newfoundland.

Year	Number of spray blocks	Spray aircraft ^a	Total gross area treated (ha) ^b
1999	1	Bell Helicopter 206	3
2000	3	Cessna 188	81
2001	3	Cessna 188	821
2002	3	Cessna 188	5000
2003	3	Cessna 188	5000
2004	4	Cessna 188 and Air Tractor 802	5000
2005	3	Cessna 188	5000

^aFixed-wing aircraft (Cessna 188 and Air Tractor 802) were equipped with Micronair AU4000 rotary atomizers. The Bell helicopter was equipped with TeeJet nozzles.

^bThe target application rate was $1-3 \times 10^9$ OBs/ha in a volume of 2.5 L/ha

With no control methods available to suppress epidemic populations of balsam fir sawfly, significant efforts were focused on determining the natural control complex of this insect and the factors responsible for the natural decline of outbreak populations (Moreau 2004). These studies indicated that all but one of the major parasitoids or predators recovered from balsam fir sawflies in western Newfoundland were generalist species, making them questionable choices as bio-control agents (McEvoy 1996). The only exception was *Cirrospilus vittatus* Walker, a specialist egg parasitoid (Huber and Moreau 2003); however, it did not appear to be a good candidate for biological control because of its low impact on populations at intermediate to high host densities (e.g., between 10 and 450 larvae per m² of foliage). Conversely, population studies revealed that an NPV (NeabNPV) exhibiting lag-density dependence always contributed to the high mortality associated with population decline (Moreau 2004). There have been many accounts of NeabNPV epizootics in previous declining balsam fir sawfly populations (Steinhaus 1949, Brown 1951, Cumming 1954, Struble 1957, Carroll 1962, Martineau 1985, West *et al.* 1992).

In parallel with population studies, NeabNPV was isolated and mass reared from natural populations of western Newfoundland (Anon. 1999, Lucarotti *et al.* 2007), initially in the laboratory and later through the treatment of field populations (Moreau *et al.* 2005). The potential of NeabNPV for controlling its host had previously been examined in Ontario (Olofsson 1973) using isolates of NeabNPV that originated from different strains of this sawfly than that observed in Newfoundland (Knerer and Atwood 1973). Nevertheless, this previous work illustrated the potential of NeabNPV for the control of its host. However, Olofsson (1973) carried out his study at a single location, in a single year, using a mistblower, in contrast to the operational scale required for the suppression of the infestation of over 50 000 ha of forest in western Newfoundland. Therefore, a trial pest management program was developed to examine the potential of NeabNPV to suppress balsam fir sawfly populations at different stages of an outbreak, while fulfilling the requirements of the Pest Management Regulatory Agency of Health Canada for efficacy and environmental safety prior to baculovirus registration (Anon. 2001). Briefly, NeabNPV, partially purified from

infected balsam fir sawfly cadavers, was applied aerially in a 20% aqueous solution of molasses to a series of blocks in sawfly-infested stands (Table 2) at a target application rate of $1-3 \times 10^9$ OBs/ha in a volume of 2.5–3.0 L/ha. The dosage was determined from available literature on aerial applications to forests (reviewed in Entwistle *et al.* 1990, Payne 2000). Control blocks (located in the same general area as the spray blocks, but at least 1 km away from spray operations) were used to determine treatment effects and the stage of outbreaks in local populations. Sawfly populations were sampled intensively before and after the spray, as well as in the years following the spray program.

Results from the initial years of this program indicate that aerial applications of NeabNPV may efficiently suppress increasing or peaking population outbreaks of the balsam fir sawfly at rates as low as 1×10^9 OBs per hectare (Moreau *et al.* 2005). In comparison, attempts to use NPVs operationally to suppress Lepidoptera populations have been conducted at application rates one to four orders higher (Cunningham and Kaupp 1995). NeabNPV applications had no appreciable effect on the generation targeted by the spray program. Nevertheless, when applied against outbreaks that were in the initial, increasing phase, a ca. 90% decline in sawfly density was observed in the generation following the aerial application of NeabNPV. When directed against peaking population outbreaks, a ca. 66% decline in sawfly density was observed in the generation following the aerial application. However, when spray applications were directed against declining populations, NeabNPV applications apparently did not influence (i.e., hasten) the natural collapse of outbreaks. Thus, outbreak stage, which can be easily deduced by sampling populations and comparing with existing data (Moreau *et al.* 2006), appeared to be a decisive variable in the understanding of the potential of NeabNPV for biological control of balsam fir sawfly. A preliminary examination of treatment effects on tree growth loss and defoliation suggests that applications targeting increasing populations reduced sawfly impacts on growth loss and stand recovery following the outbreak, whereas applications carried out at later stages of outbreaks had little or no effect on resource protection (Moreau *et al.* unpublished results).

Given that NeabNPV has no effect on non-target organisms other than sawflies (Lucarotti *et al.* 2006), it provides a safe and effective tactic to suppress epidemic populations of the balsam fir sawfly. In April 2006, NeabNPV was granted conditional registration in Canada under the trade name “Abietiv” (Lucarotti *et al.* 2006, 2007).

Pine false webworm

The pine false webworm (*Acantholyda erythrocephala* L.) was accidentally introduced into the United States in the early part of the 20th century (Wells 1926); it later spread to Canada, where it was first reported in Ontario in 1961 (Eidt and McPhee 1963). The principal hosts of the pine false webworm in central and northern Europe, its natural habitat, are Scots (*Pinus sylvestris* L.) and Austrian (*P. nigra* Am.) pines (Middlekauff 1958). In Canada, it mainly attacks red (*P. resinosa* Ait.), eastern white (*P. strobus* L.), jack (*P. banksiana* Lamb.), and Scots pines (Asaro and Allen 2001). The pine false webworm can attack both young and mature trees

(Howse and Applejohn 1993) and has caused sufficiently severe defoliation in some Ontario plantations to require salvage harvest programs (Hall 1996, Hall *et al.* 1998).

A few studies have been carried out to examine the potential of different biocontrol agents to suppress pine false webworm populations. Thus far, some positive results have been obtained with inundative releases of *Trichogramma* spp. (Bourchier *et al.* 2000) and applications of neem seed extracts (Thompson *et al.* 2003, Moreau *et al.* unpublished results). Conversely, efforts to identify and use a sex pheromone as an attractant have not been successful (Lyons and Jones 2000). Kenis and Kloosterman (2001) have also examined the potential of European parasitoids of the pine false webworm for introduction into North America. One of the most promising European parasitoids identified for classical biological control, the tachinid larval parasitoid *Myxexoristops hertingi* Mesnil, has been released in some Ontario pine forests (Kenis and Marcombe 2004), but results are not yet available.

There has been no report of a baculovirus in indigenous or introduced populations of the pine false webworm. A pest management program was developed by the Canadian Forest Service to examine the potential of NeabNPV, a baculovirus isolated from the balsam fir sawfly in Newfoundland, for biological control of the pine false webworm. Ingestion of baculovirus OBs from a baculovirus has been known to sometimes induce mortality in closely related species (e.g., Gröner 1986, Doyle *et al.* 1990, Hostetter and Puttler 1991, Cory 2000). Indeed, laboratory bioassays using a droplet-feeding method (van Frankenhuyzen *et al.* 1997) indicated that the ingestion of a dose of 1×10^6 OBs of NeabNPV by third-instar larvae of the pine false webworm reduced survival to the ultimate larval stadium by 51% compared with larvae that had ingested water only (Lucarotti *et al.* 2006). Therefore, a ground spray trial using a mistblower was conducted in a 1-ha red pine plantation located in Ontario in 2002. NeabNPV was applied in a 20% aqueous solution of molasses at rates of 0 (control), 1×10^7 , 1×10^8 , and 1×10^9 OBs per hectare on individual trees infested by the pine false webworm. In field trials, survival to the ultimate larval stadium was reduced by 57% on average, but population reduction was not associated with significant levels of foliage protection (Moreau *et al.* unpublished results). Low field efficacy of NeabNPV against the pine false webworm was attributed to three factors (i) too low a dose; (ii) absence of a replication cycle in larvae, as suggested by the absence of a carryover effect of the baculovirus the following year; and (iii) specific traits in the insect life history that hindered dose acquisition, such as larvae feeding solitarily in webbing tunnels. In 2003, seven blocks (total area of 232 ha) located in the Simcoe and Grey counties of south-central Ontario were treated with NeabNPV at an application rate of 1×10^9 OBs in 2.5 L, 20% aqueous molasses per hectare using a Cessna 188 fixed-wing aircraft. However, treatment effects were inconclusive because of inadequate control blocks and because, as was later determined, both increasing and declining populations had been targeted during trials. Nevertheless, this study may be the first field trial of a baculovirus indigenous to Canada (i.e., a baculovirus isolated from a native insect of Newfoundland) against an exotic insect species in

forests, and in this regard, promising fundamental knowledge on baculovirus cross-inoculation and mechanisms of pathogenicity in sawflies has been gained in the attempt.

The Future of Baculoviruses as Biocontrol Agents of Eruptive Forest Insects

The availability of inexpensive and effective products such as *Bt* and chemical insecticides has impeded the development of baculoviruses as biocontrol agents of forest insects. However, environmental issues and the absence of developed control tools for certain species have forced and will continue to force forest managers to seek alternative means for population suppression in many insect–host systems. It has been estimated that about 30% of the major insect pest species could be controlled by baculoviruses (Falcon 1978) and thus, the future for baculovirus research should be bright. Moreover, the development of modern recombinant techniques has defined a new horizon for baculovirus research, as these techniques may permit the development of more potent baculoviruses (Doyle *et al.* 1990, Thiem 1997, Inceoglu *et al.* 2001). However, we can expect that regulatory agencies and the general public will be hostile to the development and use of recombinant viruses in the field.

Under some conditions, baculoviruses have the potential to drive insect population fluctuations (Anderson and May 1980, Myers 1988, Dwyer *et al.* 2004); therefore, they merit a special consideration in biological control programs, at least in those systems where they dominate the dynamics of their hosts. The balsam fir sawfly/NeabNPV is a good example of such a system, whereas the spruce budworm/CfMNPV appears to be an example of a system in which baculoviruses play a very limited role (Morris 1963, Lucarotti *et al.* 2004, Moreau 2004). Accordingly, research conducted with NeabNPV (Olofsson 1973, Moreau *et al.* 2005) and CfMNPV (reviewed in Cunningham and Kaupp 1995) has been either very effective or largely unsuccessful, respectively. Therefore, if only because of the cost associated with the development, registration, and production of viral control agents, research on the use of baculoviruses for biocontrol of forest insects should concentrate on those systems where virus impact on host population is significant (Lucarotti 1997). In this regard, the criteria defined by Orlovskaya (1998) that were cited earlier may permit the identification of the most promising species. Based on the results herein, we caution that the examination of the effects of baculoviruses on eruptive populations should consider the outbreak stage as a factor that may modulate the response of populations to spray applications.

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