Prevalence of baculoviruses in spruce budworm (Lepidoptera: Tortricidae) populations in New Brunswick

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Abstract—Outbreak and declining populations of spruce budworm (*Choristoneura fumiferana* (Clem.)) were sampled extensively at three locations in New Brunswick, Canada, between 1982 and 1992 and were examined for the prevalence of granulosis and nuclear polyhedrosis viruses (Baculoviridae). Larvae, pupae, and adults were collected using a variety of methods. Spruce budworm nuclear polyhedrosis virus (*Cf*MNPV) genomic DNA probes and wet-mount light microscopy were used to determine *Cf*MNPV prevalence in 50 274 juvenile spruce budworms. Spruce budworm granulosis virus (*Chfu*GV) genomic DNA probes were used to determine the prevalence of *Chfu*GV in 25 703 of these same samples. The prevalence of both viruses was low, with *Chfu*GV and *Cf*MNPV not found in more than 15% and 2%, respectively, of samples in any collection in a given year. Prevalence of *Chfu*GV was greatest in mid- to late June in sixth-instar larvae. Each virus was detected in only two of 2177 female moths and in none of the 420 male moths examined. In the entire collection, cytoplasmic polyhedrosis virus (Reoviridae) was detected in only two budworm larvae and entomopoxvirus (Poxviridae) was not detected in any.

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Résumé—Des populations épidémiques et en déclin de la tordeuse des bourgeons de l'épinette (TBE) (*Choristoneura fumiferana* (Clem.)) situées à trois endroits au Nouveau Brunswick, Canada, ont été échantillonnées à grande échelle entre 1982 et 1992 et ont été examinées pour évaluer la prévalence des virus de la granulose et de la polyhedrose nucléaire (Baculoviridae). Les larves, les chrysalides et les adultes ont été récoltés par une variété de méthodes. Des sondes d'ADN génomique du virus de la polyhedrose nucléaire de la TBE (*Cf*MNPV) ainsi que des frottis d'insectes ont été utilizés pour déterminer la prévalence du *Cf*MNPV chez 50 274 individus juvéniles de la TBE. Des sondes d'ADN génomique du virus de la granulose de la TBE (*Chfu*GV) ont été utilisées pour déterminer sa prévalence chez 25 703 de ces

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mêmes échantillons. La prévalence des deux virus était faible, ne dépassant pas 15% pour *Chfu*GV et pas plus de 2% pour le *Cf*MNPV, pour chaque méthode d'échantillonage, pour chaque année d'étude. La prévalence du *Chfu*GV atteint son maximum entre le milieu et la fin de juin, au sixième stade de développement larvaire. Chaque virus n'a été détecté que chez deux des 2177 adultes femelles et chez aucun des 420 adultes mâles examinés. Parmis tous les specimens recueillis, le virus de la polyhèdrose cytoplasmique (Reoviridae) n'a été détecté que dans deux larves de la TBE alors que l'entomopoxvirus (Poxviridae) ne l'a été dans aucun.

Introduction

The spruce budworm (SBW) (*Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae)) is a native pest of balsam fir (*Abies balsamea* (L.) Mill. (Pinaceae)), white spruce (*Picea glauca* (Moench) Voss (Pinaceae)), red spruce (*Picea rubens* Sarg. (Pinaceae)), and black spruce (*Picea mariana* (Mill.) B.S.P. (Pinaceae)) in North America (Greenbank 1963). Outbreak populations of SBW have occurred in New Brunswick every 35 years, on average, over the last 270 years (Royama 1984). The province-wide outbreak that occurred in the 1950s was studied by researchers involved in the Green River Project (1945–1972) (Morris 1963*a*). These researchers studied the impact of mortality factors including parasitoids (Miller 1963*b*), predators (Loughton *et al.* 1963; Mook 1963; Morris 1963*b*), and disease (Neilson 1963), and Royama (1984, 1992) reanalyzed these life-table data. In that SBW outbreak, baculovirus (granulosis virus (GV) and nuclear polyhedrosis virus (NPV)) prevalence in (Neilson 1963) and impact on (Royama 1984, 1992) the population were found to be low.

The most recent SBW epidemic in New Brunswick began in 1968 (Royama 1992) and ended in the late 1980s to early 1990s. In a life-table study carried out between 1981 and 1993, outbreak and declining populations of SBW were sampled extensively. Details of the sampling methods and analyses of much of the data collected will be presented at a later date. Here, we present information on the prevalence of SBW GV (*Chfu*GV), SBW NPV (*Cf*MNPV), and other viruses in these populations.

Materials and methods

Sampling plots

Two plots at the Acadia Research Forest (plot 1, 66°16′W, 46°00′N, 1981–1989; plot 2, 66°24′W, 46°00′N, 1986–1992) about 23 km northeast of Fredericton, New Brunswick, and one plot near Saint-Quentin in northern New Brunswick (plot 3, 67°15′W, 47°29′N, 1988–1993) were sampled. Plots 1 and 2 have been described in detail (respectively, Red Pine Brook and Peter Brook in Lethiecq and Régnière 1988). In 1985, plot 1 was a closed, 35-year-old balsam fir stand with trees 9–15 m tall; in plot 2, the trees were up to 12 m tall and approximately 28 years old (Lethiecq and Régnière 1988). In plot 3 in 1990, the balsam fir trees were approximately 55 years old and up to 18 m tall.

Branch samples

One mid-crown branch was taken from each of 20 preselected, dominant or codominant balsam fir trees on each plot. These same trees or ones immediately adjacent to them were sampled on each sampling date. Collections of larvae and pupae were made at the beginning of second-instar emergence from hibernacula (roughly 10 May) until 100% moth eclosion (roughly 30 July). Between these dates, plots 1 and 2 were sampled each weekday (Monday through Friday) and plot 3 was sampled each Monday

and Thursday in each sampling year. Preemergence samples of one (during epidemic population years) or two (during endemic population years) mid-crown branches from each of these trees were also taken in mid-March of each sampling year.

Dead SBW larvae and pupae on sample branches were recorded, removed, and individually placed in gelatin capsules.

Canopy traps

Two pyramidal canopy traps were suspended 2 m above the forest canopy from each of three scaffold towers in plot 1 from 1982 to 1987. The traps were 1 m^2 and open at the base. Moths in exodus flight would enter through the open base and crawl to the top of the trap where they were caught in a 500-mL mason jar containing a piece of Vapona® (2,2-dichlorovinyl dimethyl phosphate).

Drop trays and funnels

Crown drop trays were supported by the same three scaffold towers that held the pyramidal canopy traps in plot 1. A drop tray was placed in each of the upper, middle, and lower crowns in each tower. These trays were in place between 1983 and 1987. Ten ground drop trays were distributed across plots 1 (1984–1988) and 2 (1986–1988). Canopy and ground drop trays were wood-frame squares (0.25 m^2) covered on the bottom with a fine cloth mesh. The top of each drop tray was covered with 1.2-cm galvanized metal mesh and the top edge of each frame was coated with petroleum jelly to prevent the entry of predators and scavengers. Twenty conical aluminum funnels, 45 cm in diameter at the top, were suspended 2 m from the ground by ropes beneath 20 balsam fir trees distributed across plot 3 between 1990 and 1993. Each funnel narrowed to a 1-L plastic bottle with a fine mesh bottom to retain insects but allow water to flow out. Ground drop trays and funnels were placed underneath nonsampled balsam fir trees. Traps and funnels were examined on each collection date and emptied as required. Insects caught in the traps or funnels were placed in numbered 1.5-mL microcentrifuge tubes.

Rearing

Larval emergence from preemergence sample branches was induced in the laboratory by first wrapping each individual branch (or pair in endemic population years) with unbleached paper towel and then suspending each branch about 1 m off the floor by the basal end. Branches were soaked thoroughly every morning and evening with tap water prior to the initiation of emergence and only in the evening after emergence. Laboratory conditions were maintained at 25 °C and 50%–60% RH under artificial light (16L:8D) (Strongman *et al.* 1997). Every day, while the laboratory lights were on, SBW larvae were picked up with fine-tipped paintbrushes as they climbed out of the foliage onto the branch base, paper towel, or the string suspending each branch. All larvae were individually reared in 2-dram (1 dram = 3.7 cm^3) glass vials containing SBW diet (McMorran 1965) under the same conditions described above. Branches were left hanging until no larvae had emerged for 5 consecutive days.

On each sampling date and from each plot, a single branch was selected and all SBWs found on that branch were taken for rearing. If there were fewer than 100 SBWs on the branch, additional branches were selected and all SBWs from those branches were reared to obtain a minimum of 100 SBWs per collection day and plot. Larvae and pupae that died in rearing were individually placed in numbered 1.5-mL microcentrifuge tubes.

Monitory collections

Fifty living SBW larvae and (or) pupae were arbitrarily picked from one branch from each plot on each sample day, placed individually in gelatin capsules, and frozen. Larval stadia were determined by head capsule measurement (McGugan 1954). (First, second, third, fourth, fifth, and sixth instars are referred to as L1, L2, L3, L4, L5, and L6, respectively.)

Diagnostics

Dead juvenile insects in rearing were stored at 4 °C until initial diagnoses could be made, which was usually <3 days from death. Insects that were dead at collection and adults from canopy traps were stored at –20 °C. Dead juveniles were first inspected for external signs of parasitism using a dissecting microscope. If parasitism was not suspected, dead SBWs (including monitory collections) were prepared as wet-mount squashes for microscopic examination for the presence of microorganisms (Strongman *et al.* 1997). A cross-sectional segment was cut through the mid-abdomen of the larva so that cuticle, fat body, and midgut would be included in each wet mount. Wet mounts of pupae and adults were also made from the abdomen. Following examination for disease, the remaining tissue (excluding the smeared tissue) was returned to its microcentrifuge tube and stored at –20 °C.

Prevalence of *Chfu*GV and *Cf*MNPV in SBW collections was determined by DNA dot-blot hybridization assays using whole DNA probes labelled with horseradish peroxidase and detected with enhanced chemiluminescence (ECL™, Amersham Biosciences) (Kaupp and Ebling 1993). Briefly, individual insects were macerated in an equal volume of sterile distilled water in 1.5-mL microcentrifuge tubes. A 20-µL aliquot of each macerate was placed on pretreated Hybond™-N+ nylon membrane (Amersham Biosciences) using a 96-well vacuum dot-blot apparatus (Bio-Rad). Viral DNAs were isolated, labelled with horseradish peroxidase, and immediately used as probes. After addition of the probes, the presence of virus-positive samples on the nylon membranes was recorded on Hyperfilm™ ECL™ (Amersham Biosciences) radiography film. Purified *Chfu*GV and *Cf*MNPV DNA were placed in one well each per membrane as positive controls (Kaupp and Ebling 1993).

Results

Epidemic SBW populations started to collapse in 1986 in plots 1 and 2, and in 1992 in plot 3. The prevalence of both *Chfu*GV and *Cf*MNPV in these populations was low (Table 1). The two probes used do not cross-react (Kaupp and Ebling 1993), and no joint *Chfu*GV and *Cf*MNPV infections were observed. *Chfu*GV-positive insects made up no more than 15% of dead SBWs in any collection category, and *Cf*MNPV positives made up no more than 2% of dead SBWs (Table 1). Only SBWs probed for *Chfu*GV (28 300 immature and 2597 adult SBWs) were included in the determination of the prevalence of this virus because even patent *Chfu*GV infection cannot be discerned with confidence using only wet mounts. Probing data showed that the majority of *Chfu*GV-positive larvae were collected as L6 (Fig. 1) and died as L6 (Fig. 2). The number of *Cf*MNPV positives was so low in the first 20 732 SBWs probed that probing was not carried out on additional samples. Microscopic examinations were, however, completed on all samples. Although a trend was detected among *Chfu*GV positives (Figs. 1 and 2), no trend could be detected among *Cf*MNPV positives owing to the low number in any given year and plot; however, by combining data from all plots, years, and sample methods (excluding monitory collections and canopy traps), the percent distribution of

TABLE 1. Microscopic examination and molecular probing for *Chfu*GV and *Cf*MNPV of *Choristoneura fumiferana* collected in New Brunswick between 1982 and 1992.

		Molecular probing		Microscopic examination
	Number of insects	% *Chfu*GV positive	% *Cf*MNPV positive	% *Cf*MNPV positive
Canopy traps (adults)				
Plot 1				
1982 (females)	886	0	0.11	
1983 (females)	719	0	0	
1985 (females)	572	0.35	NP	0.17
1985 (males)	420	0	0	
Subtotals*	2 597	0.08	0.08	
Crown drop trays				
Plot 1				
1984	137	0	NP	0
1985	198	4.04	0	
Ground drop trays				
Plot 1				
1985	527	2.66	NP	0
1987	182	3.85	0	
Plot 2				
1987	97	0	0	
Funnels				
Plot 3				
1990	47	12.77	NP	0
1991	134	8.21	NP	0
1992	229	3.93	NP	0
Subtotals for drop trays and funnels*	1 551	3.55	0	
Dead on branches				
Plot 1				
1985	1 428	7.35	0.98	
Plot 3				
1988	674	0.15	0.15	
1989	1 152	2.95	0	
1990	1 549	9.3	NP	0.06
1991	2 110	6.26	0.09	
1992	2 277	10.98	0.22	
Subtotals*	9 190	7.25	0.25	
Died in rearing				
Plot 1†				
1982	575	NP	NP	0.35
1983	2 042	NP	NP	0.2
1984	1 687	NP	NP	1.01
1985	9 550	NP	NP	0.37
1986	4 186	NP	NP	1.53
1987	1 926	NP	NP	2.65
1988	169	NP	NP	0

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TABLE 1 (*concluded*).

	Number of insects	Molecular probing		Microscopic examination
		% *Chfu*GV positive	% *Cf*MNPV positive	% *Cf*MNPV positive
1989	21	NP	NP	0
Plot 2[†]				
1986	2 776	NP	NP	0.61
1987	1 508	NP	NP	0.33
1988	71	NP	NP	0
1989	19	NP	NP	0
1990	41	NP	NP	0
Plot 3				
1989	977	0.41	NP	1.23
1990	1 303	7.37	1.15	
1991	2 306	8.02	0.09	
1992	1 363	14.0	0.22	
Subtotal for *Chfu*GV[‡]	5 949	8.22		
Subtotal for *Cf*MNPV*	30 520		0.74	
Monitory				
Plot 1				
1983	1 010	0.2	NP	0.1
1984	1 260	0.48	NP	0
1985	1 646	1.88	0.73	
1986	1 159	1.81	NP	0.09
1987	1 136	2.29	0.53	
Plot 2				
1986	823	0	0	
1987	1 141	0	0	
Plot 3				
1988	734	0	0	
1989	416	0.24	NP	0
1990	544	1.47	NP	0
1991	852	0.7	NP	0
1992	889	3.37	NP	0
Subtotals*	11 610	1.13	0.17	
Totals *Chfu*GV[‡,§]	28 300	4.74		
Totals *Cf*MNPV*,[§]	52 871		0.51	

NOTE: NP, not probed.

* Totals include *Cf*MNPV positives identified by both molecular probing and microscopic examination.

† Samples processed prior to the availability of molecular probes.

‡ Totals include only probed insects.

§ Totals do not include subtotals for canopy traps (adults).

dead *Cf*MNPV-positive juvenile SBWs by stadium was 2.8 for L2, 9.5 for L3, 16.9 for L4, 18.0 for L5, 21.3 for L6, and 31.5 for pupae. Of the 2597 moths probed or examined microscopically, positives were recorded for only two female moths for each virus (Table 1).

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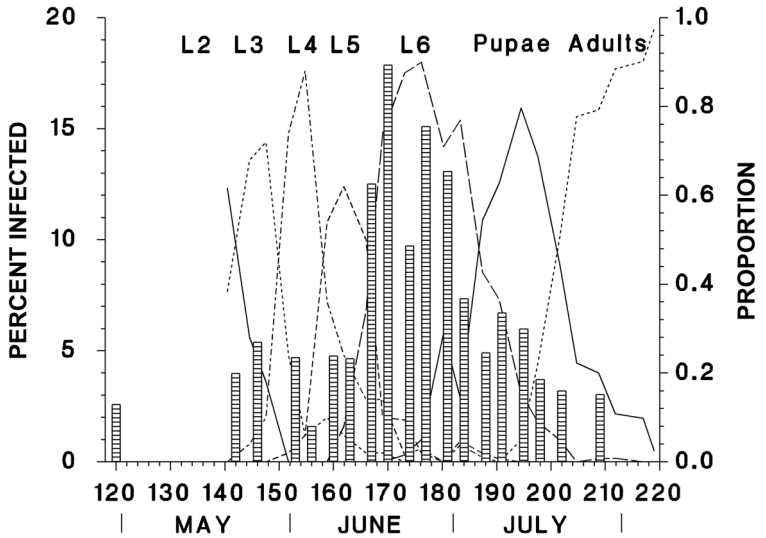

FIGURE 1. Percentage of the total number of *Choristoneura fumiferana* reared on each collection day (days of year and months) in 1992, plot 3, that died because of *Chfu*GV. Curves in the background show mean phenology for each stadium from L2 to adult over the same seasonal period.

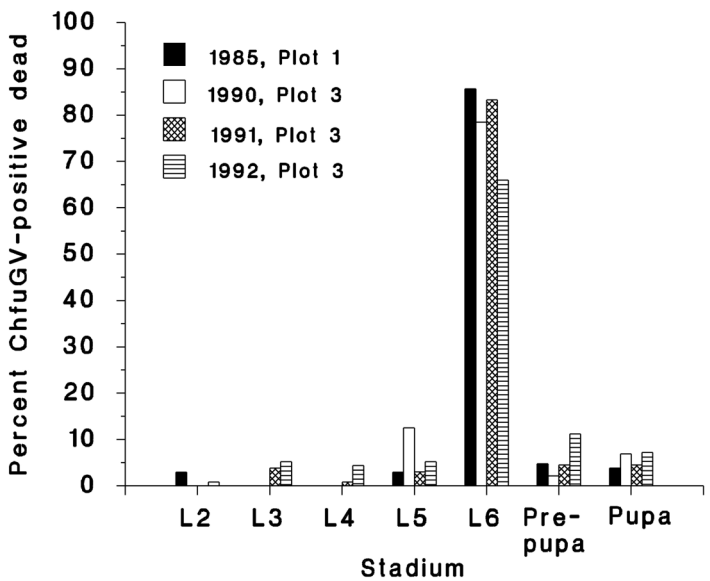

FIGURE 2. Percent distribution of stadia of *Chfu*GV-positive *Choristoneura fumiferana* collected dead from branches.

The presence of other viruses was also recorded during the course of microscopic examinations. A cytoplasmic polyhedrosis virus (Reoviridae) was found in only two SBW larvae from plot 2 (one in 1986 and one in 1988). Entomopox virus (Poxviridae) (Yuen *et al.* 1990) was not found in any SBW.

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Discussion

In the most recent SBW outbreak in New Brunswick, the prevalence of *Chfu*GV and *Cf*MNPV was found to be low across plots studied, sampling methods used, and stages of the population cycle. At Green River between 1954 and 1958, Neilson (1963) found *Chfu*GV to be the most prevalent virus but its prevalence did not exceed 15.2%, similar to our results. Furthermore, *Cf*MNPV and cytoplasmic polyhedrosis virus were found to be "so rare that it is doubtful that they have a measurable effect on budworm populations". At this point, however, it is not possible to say what the impact of either *Chfu*GV or *Cf*MNPV is on the population dynamics of SBW. In our study, the prevalence of both viruses was observed to be greatest in the late instars and pupae. Royama (1992) has concluded that decreasing survival in these life stages determines the declining trend in generational survival in SBW populations and that the contribution of mortality factors with low prevalence to the overall population dynamics cannot be evaluated without considering that contribution within the process as a whole (Royama 2001).

Baculoviruses are transmitted through ingestion by a suitable insect host. For example, in the case of the gypsy moth (*Lymantria dispar* (L.) (Lepidoptera: Lymantriidae)) NPV (*Ld*MNPV), infection can occur when a larva chews its way out of an egg whose surface has been contaminated by the female moth at the time of oviposition or through later environmental contamination (Doane 1975). *Ld*MNPV can also survive in the soil and leaf litter for years, on tree trunks for months, and on foliage for days (Podgwaite *et al.* 1979). Early gypsy moth instars may pick up *Ld*MNPV infections as they cross the forest floor; early gypsy moth instars acquire *Entomophaga maimaiga* Humber, Shimazu *et* Soper (Zygomycetes: Entomophthorales) infections in this manner (Weseloh and Andreadis 1992). In a given season, *Ld*MNPV infection of gypsy moth larvae is bimodal (Woods and Elkinton 1987): infection of the early instars is vital to infection of the later instars and the development of an epizootic. In SBW, however, the potential for early instars to encounter and become infected by *Chfu*GV or *Cf*MNPV may be limited. SBW egg masses are laid on the surface of needles, so contamination of the egg surface with *Chfu*GV or *Cf*MNPV by the female moth or through environmental contamination and subsequent ingestion of virus by emerging L1s is a possibility. Nevertheless, only four female moths were found to be positive for virus: two were positive for *Chfu*GV and two were positive for *Cf*MNPV. These positives may have been due to infection or contamination. Either way, female moth to egg transmission of either virus would likely be low, if it occurs at all (Kukan 1999). L1s may also consume viruses while ingesting waxes and other materials on the surface of needles (Retnakaran *et al.* 1999). This imbibing period would be short because L1s disperse, seek overwintering sites, and spin hibernacula soon after emergence and molt to L2 during August (Miller 1963*a*). In late April to early May of the next year, L2s disperse and mine into 1- or 2-year-old needles (McGugan 1954), molt to L3, and then wait for bud break. A low percentage of dead juvenile SBWs that were positive for either *Chfu*GV or *Cf*MNPV were L2 or L3, indicating that L1s might acquire viral infections in nature, as suggested by Retnakaran *et al.* (1999). After leaving the needles, L3s tunnel into the expanding buds where the L3s through L6s feed individually in their tunnels (Miller 1963*a*). Where larval densities are high, L5s and L6s may be forced to feed on older foliage. Thus, the later instars are probably the most likely to ingest virus and become infected. *Chfu*GV infection was more frequent than *Cf*MNPV infection, and most of the *Chfu*GV-positive dead larvae were L6. The percent distribution of all of the *Cf*MNPV-positive dead increased progressively with each stadium. To become infected, L5s and L6s require more *Cf*MNPV polyhedral occlusion bodies (OBs) than do L3s and L4s (Kaupp and Ebling 1990). There may be differences in the virulence of *Cf*MNPV

strains (Kaupp and Ebling 1990; Ebling *et al.* 1998), and *Cf*MNPV infection may develop more slowly than that of other NPVs (Barrett *et al.* 2000). Forté *et al.* (1999) reported that the LD_{50} of *Chfu*GV against L4 SBW is 5.7×10^5 OBs/larva. This is higher than the LD_{50} of *Cf*MNPV for the same instar (1.1×10^3 OBs/larva) (Kaupp and Ebling 1990). Forté *et al.* (1999) suggested that this difference may be due not so much to differences in virulence but rather to structural differences between GVs, which contain a single virion per OB, and NPVs, which contain many virions per OB.

Use of baculoviruses in forest pest suppression has been successful against insects that feed openly on foliage and in forests where natural viral epizootics occur (Lucarotti 1997). Some degree of population suppression through the application of NPVs has been achieved for Lepidoptera such as the gypsy moth (Cunningham and Kaupp 1995), the Douglas-fir tussock moth (*Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Lymantriidae)) (Otvos *et al.* 1995), and the whitemarked tussock moth (*Orgyia leucostigma* J.E. Smith) (West *et al.* 1987, 1989). Applications of *Cf*MNPV against SBW, however, have not been effective (Cunningham and Kaupp 1995). Kaupp *et al.* (1990) conducted aerial field trials with *Cf*MNPV where application of 3.44×10^{12} OBs/ha against emerging L2s (May 14) was followed by a second treatment of 2.3×10^{12} OBs/ha against L3s 26 days later. These two applications resulted in *Cf*MNPV infection of 22.9% of the larvae on June 30 (compared with 6.86% in the control plot) but failed to produce an epizootic or protect foliage. Application of *Cf*MNPV to western spruce budworm (*Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae)), which has a behavioral ecology similar to that of SBW but is 2000 times more susceptible to the virus, also failed to initiate an epizootic (Cunningham and Kaupp 1995). It would appear that low levels of *Chfu*GV and *Cf*MNPV transmission in SBW populations may have more to do with the behavior and ecology of SBW than with the virulence of these viruses.

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