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Aerial application of nucleopolyhedrovirus induces decline in increasing and peaking populations of *Neodiprion abietis*

G. Moreau [a], C.J. Lucarotti [a,*], E.G. Kettela [a], G.S. Thurston [a], S. Holmes [a], C. Weaver [a], D.B. Levin [b], B. Morin [a]

[a] *Natural Resources Canada, Canadian Forest Service-Atlantic Forestry Centre, P.O. Box 4000, Fredericton, NB, Canada E3B 5P7*
[b] *Department of Biology, University of Victoria, P.O. Box 3020, Victoria, BC, Canada V8W 3N5*

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Abstract

The potential role of diseases in generating population cycles has often been advocated but has received little experimental support from the field. We introduced a nucleopolyhedrovirus (NeabNPV) into field populations of *Neodiprion abietis* (Harris), the balsam fir sawfly, to determine its role in the collapse of outbreaks and examine its potential for biological control. This was accomplished through the use of aerial applications of NeabNPV on increasing, peaking, and declining populations of its host. Results indicate that *N. abietis* densities were distinctly lower in the generation following an aerial application of NeabNPV, but only when treatments were directed against increasing or peaking populations. When directed against declining populations, NeabNPV applications apparently did not influence the natural collapse of outbreaks. Although the artificial introduction of NeabNPV did not consistently affect densities of the treated generation, it had an effect on host biology in the weeks following the treatment as the incidence of NeabNPV infection increased and frass production (concomitant with larval feeding) decreased in treated areas. This study supports the hypothesis that NeabNPV epizootics initiate the decline of *N. abietis* populations. Our results also indicate that NeabNPV may provide an effective tactic to suppress increasing or peaking population outbreaks of *N. abietis* through the use of aerial applications of NeabNPV at rates as low as 1×10^9 polyhedral occlusion bodies per hectare.
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1. Introduction

Baculoviruses are double-stranded DNA viruses with circular genomes (Mayo and Pringle, 1998) ranging in size from just under 82 to 180 kb (Herniou et al., 2003; Lauzon et al., 2004). Baculoviruses have been reported exclusively from arthropods and have been successfully recovered and genome sequenced only from Diptera, Hymenoptera, and Lepidoptera (Herniou et al., 2004). Currently, the family *Baculoviridae* is divided into two diverse genera, *Granulovirus* (GV) and *Nucleopolyhedrovirus* (NPV). NPVs can be transmitted horizontally through ingestion of virions, occluded in proteinaceous occlusion bodies (OBs), by a suitable host. Sawfly NPVs are only known to infect the midgut epithelium so that, following viral replication, infected midgut cells containing OBs are sloughed off into the frass and out of the body where they can infect other host insects (Federici, 1997). Death normally occurs within one week (Federici, 1997) and, during that time, the hosts produce infective units of the disease. This combined with the gregarious nature of many sawfly species can lead to rapid horizontal transmission of the disease within populations.

* Corresponding author. Fax: +1 506 452 3525.
E-mail address: clucarot@nrcan.gc.ca (C.J. Lucarotti).

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Natural epizootics of baculoviruses have been reported in declining populations of several defoliators that exhibit cyclical population fluctuations (e.g., Campbell, 1963; Myers, 2000; Olofsson, 1987; Tanada, 1976). There has been a considerable amount of theoretical work on the possible involvement of baculoviruses in the population dynamics of forest insects (e.g., Anderson and May, 1980, 1981; Briggs and Godfray, 1995; Dwyer et al., 2004; McCallum et al., 2001; Myers, 1988). Conversely, few manipulative field experiments have been carried out to verify experimentally the assumptions and inferences on the role of baculoviruses drawn from models and from the analysis of population studies. To the best of our knowledge, the potential of baculoviruses to induce the collapse of populations at different stages of an outbreak cycle has never been examined at a population level.

As a case study of the role of pathogens in the decline of forest insect populations, we used the NeabNPV-*Neodiprion abietis* (Harris) (balsam fir sawfly; Hymenoptera: Diprionidae) system in managed balsam fir (*Abies balsamea* [L.] Mill.) forests of western Newfoundland, Canada. Based on defoliation data, *N. abietis* populations appear to cycle at 5–15-year intervals in that area (Anonymous, 1943–1996). Several authors have reported the occurrence of epizootics of a NPV (NeabNPV) in populations of *N. abietis* but have provided no data concerning its impact on sawfly populations (Brown, 1951; Carroll, 1962; Cumming, 1954; Martineau, 1985; Struble, 1957). More recently, ecological studies of *N. abietis* (Moreau, 2004; Moreau et al., unpublished) have highlighted that, among all the factors affecting the dynamics of this insect, NeabNPV was the only one that consistently exhibited lag-density dependence and contributed to high mortality associated with the decline of populations. Although *N. abietis* defoliation does not generally result in widespread tree mortality, it does cause extensive growth loss (Parsons et al., 2003; Piene et al., 2001) and leaves the tree more sensitive to damage by other defoliators (Cunningham, 1984). No method is currently available to suppress epidemic populations of this forest insect.

In this study, we tested the hypothesis that NeabNPV epizootics initiate the decline of *N. abietis* populations by increasing the incidence of NeabNPV infection in field populations through aerial applications of an isolate that originated from the same area. Applications were carried out over a number of years to comply with requirements of the Pest Management Regulatory Agency of Health Canada (Anonymous, 2001) and to enhance the probability of targeting different phases of an outbreak. Because the effects of an NPV may extend beyond the year of application (Otvos et al., 1987), populations were sampled in the year following application, in addition to the pre- and post-application sampling. This study also represented an opportunity to examine the potential of NeabNPV for biological control of *N. abietis*. Attempts to use NPVs to artificially suppress sawfly populations have often met with success and at application rates one or two orders below those used to control Lepidoptera with NPVs (see reviews by Cunningham and Kaupp, 1995; Wallace and Cunningham, 1995). Sawfly NPVs, however, are highly host specific and, for biological control, it has been necessary to develop a specific NPV for each host species of pest sawfly.

2. Materials and methods

2.1. *Neodiprion abietis* life cycle

In western Newfoundland, female sawflies deposit their eggs into current-year foliage and occasionally, into foliage of the previous year in late September/early October (Carroll, 1962). Eggs hatch in late June/mid-July and larvae begin to feed gregariously on the surface of needles. Larvae generally eat all but current-year foliage of balsam fir (Moreau et al., 2003; Parsons et al., 2003). Male larvae have five instars and complete their development within 30 days, whereas females can have five or six instars and complete their development within a period of about 35 days (Carroll, 1962). Last-instar larvae spin a cocoon directly on the foliage, pupate inside it, and emerge as adults 2–3 weeks later.

2.2. NeabNPV purification

NeabNPV was isolated in 1997 from *N. abietis* larvae collected between Stephenville (lat 48°32′N, long 58°33′W) and Corner Brook. To purify the virus, infected insects that had been stored frozen at −20 °C were thawed and homogenized in five volumes of a 0.3% sodium dodecyl sulfate (SDS) solution using a hand-held blender. The homogenate was stirred for 60 min, filtered through a plastic screen (1-mm^2 mesh), and the filtrate was set aside. The solids from the mesh were re-suspended in 0.3% SDS, homogenized, filtered, and the filtrate was set aside. This process was repeated until the filtrate flowed clear. The filtrates were then pooled, filtered twice through eight layers of cheesecloth, and centrifuged for 15 min at 9000*g*. The supernatant was discarded and the NeabNPV OB pellet was re-suspended in 0.3% SDS. Centrifugation and re-suspension were repeated until a clear supernatant was obtained. The NeabNPV OB pellet was then re-suspended in water.

As NeabNPV OBs are too small (0.5–1.0 μm) to be counted with a hemocytometer, a proportional counting method adapted from Wigley (1980) was used to quantify OB concentration. A suspension of latex beads (2.97 μm diameter, SD 0.04, Sigma Chemical #LB-30) was diluted in series and quantitated on a Hausser Hy-Lite counting chamber (Hausser Scientific, Blue Bell,

PA). Serial dilutions of the NeabNPV stock were also prepared. Ten microliters of a mixture containing 50 μL of a suspension of beads at 3×10^7 beads/mL plus 50 μL of an unknown suspension of NeabNPV OBs and 100 μL of 1% bovine serum albumin were spread on microscope slides and allowed to dry. Beads and OBs from 50 fields of view on each of four slides were counted under the 100× oil lens of a microscope. The concentration of OBs was determined as a proportion of the number of latex beads counted. This process was repeated with different dilutions of the NeabNPV dilution series. Once the mean concentration of OBs was obtained, the volume of the NeabNPV stock suspension was adjusted to a concentration of 4×10^9 OBs/mL. NeabNPV suspensions were stored in water at 4 °C to inhibit the growth of contaminating bacteria. Suspensions were generally used in the 5 months following the preparation.

2.3. NeabNPV amplification

In 1998, NeabNPV was amplified by applying purified virus (from 1997) to branches used to feed *N. abietis* larvae in 40-L vented plastic containers. NeabNPV was then purified from these sawflies by the method described in Section 2.2. From 1999 to 2002, NeabNPV was amplified by aerially applying purified NeabNPV to balsam fir stands supporting high-density populations of *N. abietis* (as per Section 2.5). Stands used as NeabNPV production sites were distinct from blocks used in field trials (Sections 2.4 and 2.5). Collections of larvae for NeabNPV amplification (to be distinguished from collection of larvae for monitoring of populations) began about 7 days after application. Larvae of *N. abietis* were collected by beating each individual tree from mid- to lower-crown with plastic leaf rakes. All fallen debris was captured on a tarpaulin placed beneath the trees. The debris was poured into 0.0255-m^3 brown, paper bags. Three 30-cm branch tips of fresh balsam fir foliage were added to each bag. To ensure high infection rates in larvae and maximize the yield in NeabNPV OBs, 3 mL of NeabNPV OBs suspended in water (1×10^7 OBs/mL) was misted onto the foliage of each bag. The bag tops were folded over, stapled shut, and kept at ambient laboratory temperatures (18–20 °C) until larval death or pupation. Dead larvae were hand-picked from the needles, placed into 50-mL centrifuge tubes, and kept frozen at −20 °C until NeabNPV purification (Section 2.2).

2.4. Field sites

For field trials of NeabNPV, five control blocks and seven treated blocks were selected in the area surrounding the city of Corner Brook (lat 48°57′N, long 57°57′W) on the west coast of Newfoundland, Canada (Fig. 1; Table 1). For each of the 3 years of field trials, variable numbers of blocks were set in accessible areas with *N. abietis* densities greater than 150 eggs/m^2 of foliage, based on surveys carried out by the Newfoundland Department of Natural Resources each winter. Block size was determined as a function of the amount of NeabNPV suspension produced through amplification (Section 2.3). Control blocks were in the same general area as spray blocks but at least 1 km away to avoid any drift from spray operations. Blocks were dominated by naturally regenerated, precommercially thinned balsam fir stands that also contained some black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss). Trees were 25–35 years old, 7.5 m tall on average, and grew at densities of 1900–2600 stems/ha. Thinning operations had been carried out more than ten years previously and the canopy was closing in all sites. According to indices in Meades and Moores (1994), blocks corresponded to typical balsam fir forests found within the Corner Brook subregion of the western Newfoundland ecoregion.

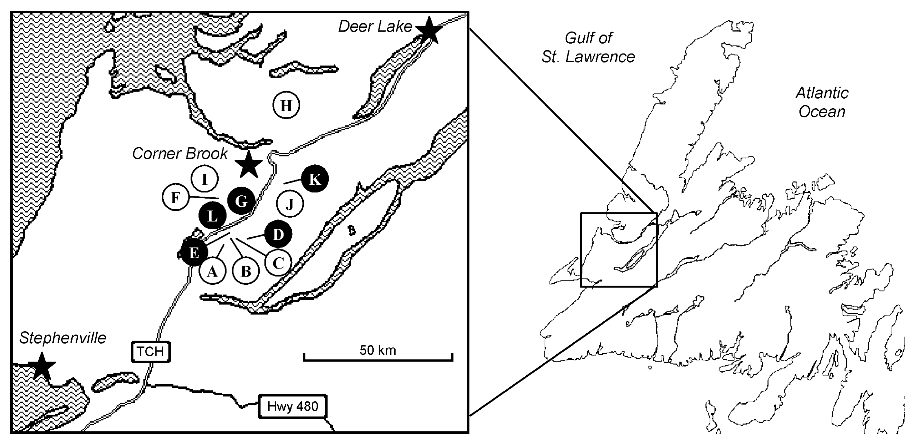

Fig. 1. Map of Newfoundland, Canada identifying the study area. Blocks are designated as A (00-T1), B (00-T2), C (00-T3), D (00-C1), E (00-C2), F (01-T1), G (01-C1), H (02-T1), I (02-T2), J (02-T3), K (02-C1), and L (02-C2) (see Table 1). White and black circles represent treated and control blocks, respectively.

Sample lines of 100 m were established close to the center of each block and away from block edges. As the reduction of population density associated with spray treatments increases the variance associated with density, one sample line was laid in each control block and three lines were laid in each treated block to maintain the accuracy of density estimates. One circular plot (~5 m in diameter) was established every 10 m along each sample line, for a total of 10 adjoining plots per line. Three balsam fir trees were selected in each plot (hereafter refer to as sample trees).

2.5. Field trials

Parameters of aerial spray applications during 2000, 2001, and 2002 are detailed in Table 1. Note that the treatment of some blocks was completed in two successive spray sessions. In all field trials, NeabNPV was applied in a 20% aqueous solution of molasses from Cessna 188 "Ag Truck" airplanes equipped with four underwing Micronair AU4000 atomizers. Micronair atomizers were set at a speed of 6000 rpm to target a spray droplet diameter of 80 μm. To reduce unwanted bacterial propagation, NeabNPV suspensions were added to the hopper on the aircraft containing the solution of molasses immediately prior to aircraft take-off for the spray site. To permit the monitoring of spray deposit on Kromekote cards and the foliage, FD&C blue #1 dye, 0.075% (Werner Jenkinson, Kingston, ON) was added to the final spray solution. The average application rate was 1–3×10^9 OBs/ha in a volume of 2.5–3.0 L/ha (ULV). Airplanes flew at a speed of 177 km/h, with a track spacing of 23–25 m and a track flow rate of 17.5–20.0 L/min. Parameters of spray applications were determined from available literature on aerial applications to forests (reviewed in Entwistle et al., 1990; Payne, 2000).

In all blocks, spray deposit was monitored by using white Kromekote cards (10 × 10 cm). One card was laid out in the center of each plot just before the aerial spray. After the spray treatment, the cards were brought back to the laboratory and the number of droplets that landed on five randomly selected areas (1 cm^2) on each card was determined. In addition, foliage was gently collected from the mid-crown of each sample tree 1 h after the spray in blocks 01-T1, 02-T2 (but not from line 2 in block 02-T2) and 02-T3 to compare foliar spray deposit with deposit on Kromekote cards. Eight shoots per tree (four shoots of current-year foliage, four shoots of 1-year-old foliage) were brought back to the laboratory and 10 needles per shoot were pressed between two glass microscope slides (25 × 75 × 1 mm). A compound microscope was used to determine the number of droplets on the adaxial (i.e., upper) surface of each needle.

2.6. Monitoring of populations

To monitor *N. abietis* densities, mid-crown branch tips (45-cm) were taken using a set of pole pruners from each sample tree within each treated and control block (i) prior to NeabNPV application, (ii) each week after NeabNPV application until pupation, and (iii) from the egg stage to the third instar of the subsequent generation in the year following the application. All 30 sample trees in a line were consistently sampled at every collection in a block. Collections in the year following the application began 1–3 weeks before balsam fir budburst. The numbers of live eggs, larvae, and cocoons present on treated and control branches were recorded. To express *N. abietis* density in terms of surface area of foliage, the branch surface area (foliated and defoliated) was estimated by multiplying the length (45 cm) and average width of branches. To assess the effect of spray treatments on

Table 1
Summary of 2000–2002 aerial spray application parameters for NeabNPV, Newfoundland

Block		Latitude	Longitude	Area sprayed (ha)	Timing of operation(s)	Temperature (°C)	RH (%)	Deposit on cards (droplets/cm²)[a]	Deposit on foliage (droplets/needle)[a,b]
A	00-T1	48°45′30.3″N	58°4′37.2″W	17.4	July 23 2000 06:10–06:25	10.0	100	3.04 ± 2.87	nd
B	00-T2	48°45′55.5″N	58°3′13.1″W	16.9	July 22 2000 21:20–21:30	11.0–13.0	90	10.04 ± 8.62	nd
					July 23 2000 05:55–06:08	9.5–10.0	100	8.54 ± 5.92	nd
C	00-T3	48°46′47.6″N	58°2′4.2″W	46.9	July 22 2000 20:45–21:18	14.0–15.0	88–90	26.98 ± 15.18	nd
D	00-C1	48°46′2.6″N	58°0′17.5″W	—	—	—	—	0.00 ± 0.00	nd
E	00-C2	48°47′7.1″N	58°2′3.5″W	—	—	—	–	0.00 ± 0.00	nd
F	01-T1	48°51′9.4″N	57°59′14.0″W	50.0	July 21 2001 06:15–06:50	12.9	72–77	3.91 ± 4.37	0.70 ± 1.82
G	01-C1	48°51′18.8″N	57°58′23.5″W	—	—	—	–	0.00 ± 0.00	nd
H	02-T1	49°3′23.3″N	57°51′45.6″W	2093.0	July 24 2002 19:50–20:40	13.3–16.4	65–83	7.93 ± 9.82	nd
					July 25 2002 07:35–08:25	6.5–10.5	97–100	7.58 ± 8.57	nd
I	02-T2	48°53′18.4″N	58°4′49.4″W	2594.0	July 21 2002 19:15–20:00	15.6–19.0	41–50	6.81 ± 6.39	0.18 ± 0.84
					July 22 2002 09:20–10:15	15.2–16.8	71–73	1.67 ± 4.67	0.14 ± 0.60
J	02-T3	48°52′13.0″N	57°52′55.9″W	313.0	July 22 2002 06:30–07:40	14.1	64–71	10.39 ± 7.89	0.26 ± 0.96
K	02-C1	48°53′12.2″N	57°53′31.5″W	—	—	—	—	0.00 ± 0.00	nd
L	02-C2	48°49′56.6″N	58°3′54.7″W	—	—	—	—	0.00 ± 0.00	nd

[a] Mean ± SEM.
[b] "nd" indicates that deposit on foliage was not determined.

NeabNPV infection, fifty individuals per block per larval collection in the year of the application were selected randomly, individually placed in 1.5-mL microcentrifuge tubes, and stored at −20 °C for probing (Section 2.7). The head capsules of a randomly selected subsample of 100 larvae per sample line per collection were measured under a compound microscope. Head capsule measurements were then compared with those of Carroll (1962) to determine the mean larval instar.

To assess the effect of spray treatments on frass production, frass collectors were laid in a limited number of stands (01-C1, 01-T1, 02-C1, 02-C2, 02-T2, and 02-T3). Ten frass collectors were laid out along sample lines 1 week before the NeabNPV application. Frass collectors consisted of a 50-cm (diameter) plastic funnel with a plastic collection jar attached at the bottom. To avoid water accumulation, the bottom of the collection jars was replaced with a 1-mm^2 mesh screen. Although rain may have removed some frass, this should not affect our analysis because comparisons are made between control and nearby treated stands, which should have received the same amount of rainfall. Frass collectors were emptied each week and were removed when the insects reached the cocoon stage. The monitoring of frass production was carried out only during the year of the spray application. Collected frass was brought back to the laboratory, oven dried, sorted to remove insects and other coarse debris, and weighed.

2.7. Probing

To detect NeabNPV infection in frozen specimens, molecular probing was carried out using Renaissance (Perkin-Elmer Life Sciences, Boston, MA, USA) labeling and detection kits. The probe was made from seven NeabNPV DNA/*Eco*R1 cloned fragments (3.5–5.5 kb), labeled using the Renaissance Random Primer Fluorescein Labeling Kit which produces labeled DNA probes that are typically between 300 and 600 bases. This kit incorporates Fluorescein-N6-dATP as it synthesizes DNA using the NeabNPV/*Eco*RI fragments as template.

Specimens to be probed were thawed and individually homogenized using sterile plastic pestles in micro-centrifuge tubes with a small volume of sterile water approximating the volume of the insect. A 3-μL aliquot of every insect sample was blotted onto Biodyne A nylon membranes, 0.45 μm (Pall, Gelman Laboratory). A positive control, either purified NeabNPV DNA or NeabNPV OBs, was also applied to each membrane. Control membranes spotted with infected and uninfected *N. abietis* and serial dilutions of NeabNPV OBs in concentrations ranging from 5×10^1 to 5×10^6 were also probed. The insects used as positive and negative controls were homogenized in sterile water and centrifuged for 5 min at 16,000*g* to concentrate the virus. The resulting pellet was examined under microscope for the presence of NeabNPV OBs.

The membranes were soaked with a denaturing solution (0.5 N NaOH, 1.5 M NaCl) and incubated for 30 min at 65 °C to dissolve the OBs, and release and denature the target DNA from the samples. The membranes were then neutralized (1.5 M NaCl, 0.5 M Tris, pH 7.0) for 1 min at room temperature, soaked in 10× SSC for 5 min and allowed to dry on filter paper. The membranes were exposed to 125 mJ of UV radiation in a UV linker to bind the DNA to the membrane. The hybridization was accomplished by incubating the membranes at 65 °C for 18 h in hybridization solution containing the labeled probe. High stringency washes then removed the excess probe and probe bound to non-specific targets. The probing and detection were accomplished according to the Renaissance detection protocol. The results were recorded on Kodak BioMax ML film. The lower detection limit for probing was 5×10^3 OBs, which implies that only specimens that had replicated the virus were detected.

2.8. Statistical analysis

Pearson correlations were performed to compare deposits on cards and on the foliage of adjacent trees. To examine the effects of spray treatments on frass production, statistical analyses were performed using a mixed-model ANOVA for a split-plot design. Blocks correspond to pairs of adjacent treated and control blocks (01-C1/01-T1, 02-C1/02-T3, 02-C2/02-T2). The variable "spray treatment" is included as a fixed effect, "block" as a random effect, and "time" as a covariate. Data from the same pairs of stands were used to perform a multiple regression to examine the effect of time and spray treatments on the incidence of NeabNPV infection. The rate of change in unsprayed populations from spray year to subsequent year was determined by subtracting the logarithm of the mean pre-spray densities in control blocks in the year of the spray (t) from the logarithm of the mean density at the same instar in the subsequent year ($t + 1$) in the same blocks. Based on contemporary studies of *N. abietis* outbreak populations carried out in the same area (Moreau, 2004), positive values of rate of change ($R \geqslant 0.5$) indicate that populations are increasing, negative values ($R \leqslant -0.5$) indicate population decline, and values close to zero ($-0.5 < R < 0.5$) indicate that populations are peaking. To determine the effects of spray treatments on: (i) population decline in the week following the aerial spray and (ii) population density in the early stages of larval development (i.e., egg to third-instar larvae) the year following the aerial spray, we compared slopes and intercepts of logistic regressions between population density and time (in days) (Dasgupta and Chen, 2002). Instar and infection estimates were block specific, frass and deposit estimates were plot specific, and density estimates were branch specific. Residuals were examined to ensure that postulates of parametric analyses were respected.

3. Results

3.1. Spray deposit

The average spray deposit on cards and foliage is shown in Table 1. Monitoring of spray deposit on cards and foliage indicated that deposit occurred in all the plots within the spray blocks. In spray blocks, deposits on cards and on the foliage of adjacent trees were correlated ($n = 20$ in 02-T2; $n = 30$ in 01-T1 and 02-T3; $0.57 \geqslant r \geqslant 0.48$; $P < 0.01$).

3.2. Effects of aerial applications on host biology

Analyses carried out using time (Julian date) or larval instar as an independent variable generated the same results because of the strong correlation between both variables ($n = 0.24$; $r = 0.96$; $P < 0.01$) (Fig. 2A). Thus, for simplicity, calculations using instar are not presented.

A multiple regression indicated that the combined effect of time and spray treatments explained 87% of the variation in NeabNPV infection in the weeks following the spray treatment (ln{NeabNPV infection} = 1.3018{spray treatment} − 0.1797{Julian date} + 0.0009{Julian date^2}; spray treatment = 1 in treated blocks and spray treatment = 0 in control blocks; $r^2 = 0.87$; $F_{3,21} = 54.71$; $P < 0.01$) (Fig. 2B). This regression shows that the incidence of naturally occurring NeabNPV infection increased with time in control blocks but increased more rapidly with time and reached higher levels in treated than in control blocks ($t_{21} = 3.43$; $P < 0.01$) (Fig. 2B). In the same period, frass production increased with time ($n = 0.24$; $r = 0.70$; $P < 0.01$) (Fig. 2C), but spray treatments induced a 31% reduction in frass production (mean amount of frass in collectors per day ± SEM: control = 752.6 ± 134.3 mg; treated = 496.9 ± 57.7 mg; $F_{1,2} = 44.48$, $P = 0.02$).

3.3. Effects of aerial applications on populations density

Data gathered in control blocks indicated that the respective populations in field trials were increasing in 2000 (positive rate of change; $R = 0.903$), peaking in 2001 (rate of change close to zero; $R = 0.031$), and rapidly declining in 2002 (negative rate of change; $R = -1.434$) at the time sprays were conducted (Fig. 3). Although some differences in the speed of population decline following the aerial spray were detected between treated and control blocks in each trial, results were not consistent, with treated populations sometimes declining faster (Blocks 00-T2 and 02-T2; $\chi^2 \geqslant 5.41$; $df = 1$; $P \leqslant 0.02$), at the same speed (Blocks 00-T1, 00-T3, 02-T1, and 02-T3; $\chi^2 \leqslant 0.54$; $df = 1$; $P \geqslant 0.46$), or slower (Block 01-T1; $\chi^2 = 5.73$; $df = 1$; $P = 0.02$) than control populations (Fig. 3). Depending on the rate of change of populations, variable results with respect to insect density were observed in the year following the aerial spray.

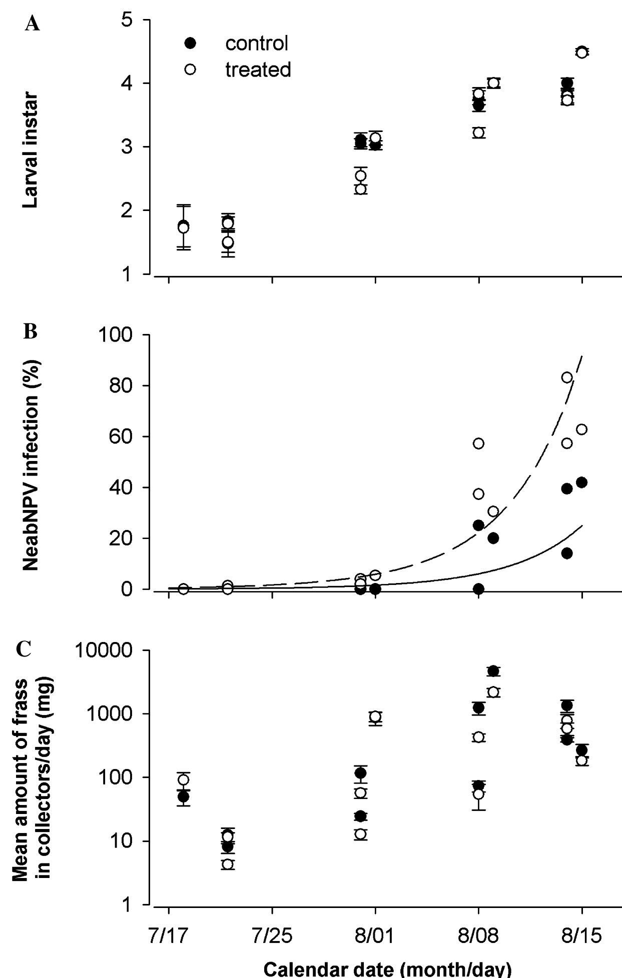

Fig. 2. Effects of time on: (A) larval instar, (B) the incidence of NeabNPV infection, and (C) frass production in control (black dots; solid line) and treated (white dots; dashed line) blocks. Trend lines for control and treated blocks in (B) were fitted using a multiple regression. Error bars correspond to ±1 SEM.

With increasing populations, as in 2000, egg-to-third-instar density was almost one order of magnitude lower in treated than in control blocks in the year following NeabNPV application ($\chi^2 \geqslant 11.44$; $df = 1$; $P < 0.01$) (Fig. 3A). With peaking populations, as in 2001, egg-to-third-instar density was half an order of magnitude lower in the treated than in the control block in the year following NeabNPV application ($\chi^2 = 41.44$; $df = 1$; $P < 0.01$) (Fig. 3B). In contrast, following application of NeabNPV to decreasing populations in 2002, egg-to-third-instar density was similar in treated and control blocks ($\chi^2 \leqslant 2.57$; $df = 1$; $P \geqslant 0.11$), except for one treated block (02-T2) that supported higher densities than all other blocks ($\chi^2 \geqslant 166.26$; $df = 1$; $P < 0.01$) (Fig. 3C).

4. Discussion

This study supports the hypothesis that NeabNPV epizootics can initiate population decline in *N. abietis* as

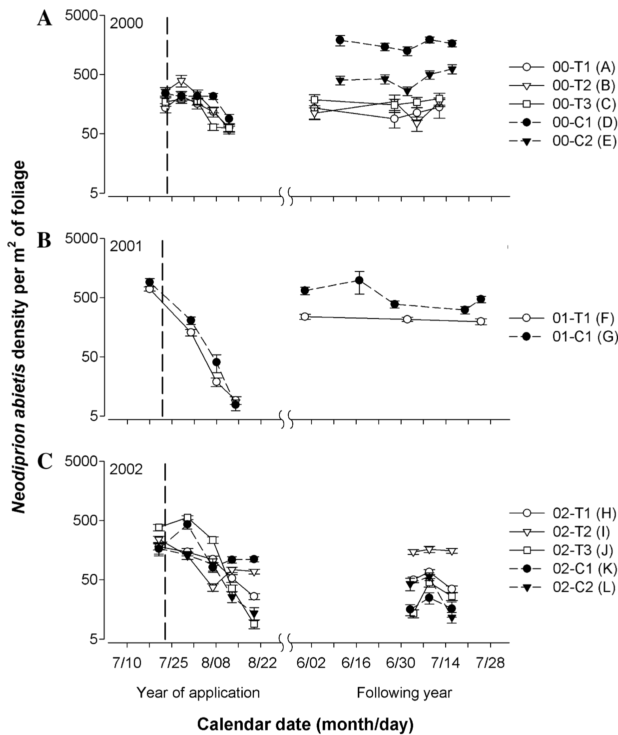

Fig. 3. Changes in *N. abietis* density (±1 SEM) from the pre-application sample to the third instar of the subsequent year in: (A) increasing, (B) peaking, and (C) declining populations. Vertical dashed lines indicate date of aerial applications.

an artificial augmentation of the incidence of NeabNPV infection induced a premature collapse of populations but only when NeabNPV applications were directed against increasing or peaking populations. In those situations, up to a 10-fold decline in *N. abietis* density was observed in treated blocks in the generation following an aerial application of NeabNPV. When directed against declining populations, NeabNPV applications apparently did not influence the natural collapse of outbreaks, with one control block even supporting higher densities than parallel controls for unknown causes. Egg density estimates gathered in plots adjacent to ours by the Forest Resources Branch of the government of Newfoundland and Labrador (Hubert Crummey, personal communication) corroborate our estimates. They also indicate that, since the time sampling was stopped, populations have declined and remained at low levels in all blocks up to the submission of this manuscript. To the best of our knowledge, this is the first report of a field introduction of a baculovirus targeting different stages of a forest insect outbreak.

The potential role of diseases in generating population fluctuations has been advocated by many authors (e.g., Anderson and May, 1980, 1981; Briggs and Godfray, 1995; Dwyer et al., 2004; McCallum et al., 2001; Myers, 1988) but thus far, their conclusions have received little experimental support from the field. There are few examples of introductions of viruses causing the collapse of forest insect populations (Orlovskaya, 1998) even though NPVs have been tested for biocontrol in both agriculture and forestry (Black et al., 1997; Inceoglu et al., 2001; Moscardi, 1999). In previous field tests carried out in forest ecosystems, baculoviruses did not induce the collapse of outbreak populations of *Operophtera brumata* (Cunningham et al., 1981) or *Choristoneura occidentalis* (Otvos et al., 1989), but did cause the premature collapse of increasing populations of *Orgyia pseudotsugata* (Otvos et al., 1987). The outbreak stage of a population is not the only factor that may explain the success or failure of field trials; complex interactions between the insect, the baculovirus, and other biotic and abiotic factors also play a role in epizootic events (Entwistle et al., 1988). Other natural enemies, although apparently not required for the induction of population collapse, may also have been involved in the decline of *N. abietis* populations. However, population-level studies of this insect (Moreau, 2004) have shown that no other NPV or microbial agent with similar effects is present in *N. abietis* populations, which simplified our diagnosis of treatment effects.

The artificial introduction of NeabNPV did not consistently affect densities of the treated generation in the initial period directly following the treatment, but it did have some effects on host biology, namely on infection rates and frass production. The incidence of naturally occurring NeabNPV infection increased with time in control blocks but increased more rapidly with time and reached higher levels in treated than in control blocks. Frass production increased with time and increasing larval instar, probably because late-instar larvae are responsible for the majority of the defoliation (Parsons et al., 2003). However, frass production, and thus presumably larval feeding, was lower in treated than in control blocks. Reduced larval feeding rates may be caused by viral damage to midgut epithelial cells, resulting in loss of vigor and a moribund state preceding death.

Mechanisms explaining how NeabNPV introduction affected the generation following the aerial application in increasing and peaking populations are not well understood and warrant additional investigations. We suspect that a combination of horizontal transmission, reduced oviposition and egg hatch (Campbell, 2002), possible vertical transmission, NeabNPV mortality in the cocoon, and emigration may be involved in the decline of treated populations. Horizontal transmission is likely to occur, as OBs can persist in the environment for a considerable time (Clark, 1956; Thompson et al., 1981). Moreover, some cadavers of *N. abietis* remain on the foliage in the year following NeabNPV epizootics (G. Moreau, personal observation) and may be an important source of infectious inoculum. Reduced oviposition due to sublethal infection, as well as vertical transovum (i.e., surface contamination of eggs) and

transovarial (i.e., contamination within the egg) transmission, has been observed in other defoliator/virus systems (Cory and Myers, 2003; Rothman and Myers, 1996). The high incidence of NeabNPV infection observed in treated blocks should result in severe mortality in the cocoon stage (not quantified here) as mortality in cocoons increases with increasing incidence of larval infection with NeabNPV (Moreau, 2004). Emigration following epizootics has been indirectly detected in *N. abietis* populations (Moreau, 2004; Moreau et al., unpublished) and may also contribute to population decline.

Our results indicate that NeabNPV applications at rates as low as 1×10^9 OBs/ha may provide an effective yet environmentally friendly tactic to suppress epidemic populations of *N. abietis*. Except for sawflies, NeabNPV has no effects on non-target organisms (Moreau et al., unpublished). Populations of *N. abietis* usually remain at outbreak densities for only 2–4 years before collapsing under the influence of natural NeabNPV (Martineau, 1985; Moreau, 2004; Smith, 1947). Thus, interventions in the initial 2 years of an outbreak should be favored to reduce the impact of *N. abietis* on the forest resource. Delaying NeabNPV treatments until the third or fourth year of an infestation (e.g., the field trials of 2002) apparently had no impact on population densities and probably little or no effect on resource protection. Conversely, a certain insect density might be necessary for horizontal transmission of NeabNPV to take place (Anderson and May, 1981; Beisner and Myers, 1999), and thus applications carried out before a sufficient buildup of populations has occurred might be ineffective.

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References

Anderson, R.M., May, R.M., 1980. Infection diseases and population cycles of forest insects. Science 210, 658–661.

Anderson, R.M., May, R.M., 1981. The population dynamics of microparasites and their invertebrate hosts. Philos. Trans. R. Soc. Lond. B 291, 451–524.

Anonymous, 1943–1996. Annual Report. Newfoundland Forest Protection Association, St. John's, NL.

Anonymous, 2001. Regulatory Directives DIR2001-02: Guidelines for the Registration of Microbial Pest Control Agents and Products. Pest Management Regulatory Agency, Health Canada, Ottawa, ON.

Beisner, B.E., Myers, J.H., 1999. Population density and transmission of virus in experimental populations of western tent caterpillar (Lepidoptera: Lasiocampidae). Environ. Entomol. 28, 1107–1113.

Black, B.C., Brennan, L.A., Dierks, P.M., Gard, I.E., 1997. Commercialization of baculoviral insecticides. In: Miller, L.K. (Ed.), The Baculoviruses. Plenum Press, NY, pp. 341–387.

Briggs, C.J., Godfray, H.C.J., 1995. The dynamics of insect–pathogen interactions in stage-structured populations. Am. Nat. 145, 855–887.

Brown, C.E., 1951. Forest insect survey notes. Can. Dep. Agri. Bi-Month. Prog. Rep. 7, 2.

Campbell, C.S., 2002. Persistence of a nucleopolyhedrovirus in populations of balsam fir sawfly, *Neodiprion abietis* Harr. (Hymenoptera: Diprionidae), following aerial applications of the virus. Undergraduate Thesis, University of New Brunswick, NB.

Campbell, R.W., 1963. The role of disease and desiccation in the population dynamics of the gypsy moth *Porthetria dispar* (L.) (Lepidoptera: Lymantriidae). Can. Entomol. 95, 426–434.

Carroll, W.J., 1962. Some aspects of the *Neodiprion abietis* (Harr.) complex in Newfoundland. Ph.D. Dissertation, Syracuse University, Syracuse, NY.

Clark, E.C., 1956. Survival and transmission of a virus causing polyhedrosis in *Malacosoma fragile*. Ecology 37, 728–732.

Cory, J.S., Myers, J.H., 2003. The ecology and evolution of insect baculoviruses. Annu. Rev. Ecol. Syst. 34, 239–272.

Cumming, M., 1954. Diseases of insects. Can. Dep. Agri. Bi-Month. Prog. Rep. 10, 3.

Cunningham, J.C., 1984. *Neodiprion abietis* (Harris), balsam fir sawfly (Hymenoptera: Diprionidae). In: Kelleher, J.S., Hulme, M.A. (Eds.), Biological Control Programmes against Insects and Weeds in Canada 1969–1980. Commonwealth Agricultural Bureaux, Slough, England, pp. 321–322.

Cunningham, J.C., Kaupp, W.J., 1995. Insect viruses. In: Armstrong, J.A., Ives, W.G.H. (Eds.), Forest Pest Insects in Canada. Natural Resources Canada, Ottawa, ON, pp. 327–340.

Cunningham, J.C., Tonks, N.V., Kaupp, W.J., 1981. Viruses to control winter moth, *Operophtera brumata* (Lepidoptera: Geometridae). J. Entomol. Soc. Br. Columbia 78, 17–24.

Dasgupta, N., Chen, G., 2002. Some robustness issues for comparing multiple logistic regression slopes to a control for small samples. J. Stat. Comput. Sim. 72, 925–935.

Dwyer, G., Dushoff, J., Yee, S.H., 2004. The combined effects of pathogens and predators on insect outbreaks. Nature 430, 341–345.

Entwistle, P.F., Cory, J.S., Doyle, C., 1988. An overview of baculovirus ecology as a background to field release of a genetically manipulated nuclear polyhedrosis virus. In: Klingmüller, W. (Ed.), Risk Assessment for Deliberate Releases. Springler, Berlin, Heidelberg.

Entwistle, P.F., Evans, H.F., Cory, J.S., Doyle, C., 1990. Questions on aerial application of microbial pesticides to forests. In: Proceedings of Fifth International Colloquium on Invertebrate Pathology, 20–24 August 1990, Adelaide, Australia, pp. 159–163.

Federici, B.A., 1997. Baculovirus pathogenesis. In: Miller, L.K. (Ed.), The Baculoviruses. Plenum Press, New York, pp. 33–59.

Herniou, E.A., Olszewski, J.A., Cory, J.S., O'Reilly, D.R., 2003. The genome sequence and evolution of baculoviruses. Annu. Rev. Entomol. 48, 211–234.

Herniou, E.A., Olszewski, J.A., O'Reilly, D.R., Cory, J.S., 2004. Ancient coevolution of baculoviruses and their insect hosts. J. Virol. 78, 3244–3251.

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

Lauzon, H.A.M., Lucarotti, C.J., Krell, P.J., Feng, Q., Retnakaran, A., Arif, B.M., 2004. Sequence and organization of the *Neodiprion lecontei* nucleopolyhedrovirus genome. J. Virol. 78, 7023–7035.

Martineau, R., 1985. Insectes nuisibles des forêts de l'Est du Canada. Service canadien des forêts, Rapport technique de foresterie 32F, Broquet, Ottawa.

Mayo, M.A., Pringle, C.R., 1998. Virus taxonomy—1997. J. Gen. Virol. 79, 649–657.

McCallum, H., Barlow, N.D., Hone, J., 2001. How should pathogen transmission be modelled? Trends Ecol. Evol. 16, 295–300.

Meades, W.J., Moores, L., 1994. Forest Site Classification Manual (Sec. Ed.). A Field Guide to the Forest Types of Newfoundland. Canada, Newfoundland Forest Resource Development Agreement, FRDA Report 003.

Moreau, G., 2004. The influence of forest management on defoliator populations: a case study with *Neodiprion abietis* in precommercially thinned and natural forest stands. Ph.D. Dissertation, University of New Brunswick, Fredericton, NB.

Moreau, G., Quiring, D.T., Eveleigh, E.S., Bauce, É., 2003. Advantages of a mixed diet: feeding on several foliar age classes increases the performance of a specialist insect herbivore. Oecologia 135, 391–399.

Moscardi, F., 1999. Assessment of the application of baculoviruses for control of Lepidoptera. Annu. Rev. Entomol. 44, 257–289.

Myers, J.H., 1988. Can a general hypothesis explain population cycles of forest Lepidoptera?. Adv. Ecol. Res. 18, 179–242.

Myers, J.H., 2000. Population fluctuations of the western tent caterpillar in southwestern British Columbia. Popul. Ecol. 42, 231–241.

Olofsson, E., 1987. Mortality factors in a population of *Neodiprion sertifer* (Hymenoptera: Diprionidae). Oikos 48, 297–303.

Orlovskaya, E.V., 1998. The theoretical basis for using baculoviruses to control forest pests. In: McManus, M.L., Liebhold, A.M. (Eds.), Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service, General Technical Report NE-247, pp. 206–212.

Otvos, I.S., Cunningham, J.C., Alfaro, R.I., 1987. Aerial application of nuclear polyhedrosis virus against Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Lymnatriidae): II. Impact 1 and 2 years after application. Can. Entomol. 119, 707–715.

Otvos, I.S., Cunningham, J.C., Kaupp, W.J., 1989. Aerial application of two baculoviruses against the western spruce budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae), in British Columbia. Can. Entomol. 121, 209–217.

Parsons, K., Quiring, D., Piene, H., Farrell, J., 2003. Temporal patterns of balsam fir sawfly defoliation and growth loss in young balsam fir. Forest Ecol. Manag. 184, 33–46.

Payne, N.J., 2000. Factors influencing aerial insecticide application to forests. Integr. Pest Manag. Rev. 5, 1–10.

Piene, H., Ostaff, D., Eveleigh, E., 2001. Growth loss and recovery following defoliation by the balsam fir sawfly in young, spaced balsam fir stands. Can. Entomol. 133, 675–686.

Rothman, L.D., Myers, J.H., 1996. Debilitating effects of viral diseases on host Lepidoptera. J. Invertebr. Pathol. 67, 1–10.

Smith, H.V.E., 1947. Annual Report. Newfoundland Forest Protection Association, St. John's, NL.

Struble, G.R., 1957. Biology and control of the white-fir sawfly. Forest Sci. 1, 196–209.

Tanada, Y., 1976. Ecology of insect viruses. In: Anderson, J.F, Kaya, H.K. (Eds.), Perspectives in Forest Entomology. Academic Press, New York, pp. 265–283.

Thompson, C.G., Scott, D.W., Wickman, B.E., 1981. Long-term persistence of the nuclear polyhedrosis virus of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae), in forest soil. Environ. Entomol. 10, 254–255.

Wallace, D.R., Cunningham, J.C., 1995. Diprionid sawflies. In: Armstrong, J.A., Ives, W.G.H. (Eds.), Forest Pest Insects in Canada. Natural Resources Canada, Ottawa, ON, pp. 193–232.

Wigley, P.J., 1980. Counting microorganisms. In: Kalmakoff, J., Longworth, J.F. (Eds.), Microbial Control of Insects Pests. NZ Dep. Sci. Ind. Res. Bull. 228, Wellington, pp. 29–35.