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Application of Balsam Fir Sawfly Nucleopolyhedrovirus against its Natural Host *Neodiprion abietis* (Hymenoptera: Diprionidae)

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Abstract – Fifty-hectare blocks of balsam fir forest, in western Newfoundland Canada, were treated with 1 - 3 x 10^9 occlusion bodies/hectare of *Neodiprion abietis* nucleopolyhedrovirus (*Neab*NPV) in 2.5 L 20% aqueous molasses using Cessna 188 'Ag Truck' airplanes equipped with Micronaire AU 4000 rotary atomizers. In the weeks following application, there was higher balsam fir sawfly larval mortality in the spray blocks than in the control. In the subsequent year, there was lower percentage egg hatch and higher larval mortality in samples collected from the spray blocks compared to those from the control block. Balsam fir sawfly pupae with white as opposed to brown pupal cases were significantly more like to harbour *Neab*NPV infection. These results suggest that aerial applications of *Neab*NPV can suppress outbreaking balsam fir sawfly populations.

I. Introduction

Since 1990, outbreak populations of balsam fir sawfly (*Neodiprion abietis* Harr.) have affected approximately 260,000 ha of high-value, precommercially-thinned, balsam fir (*Abies balsamea* (L.) Mill.) forest in western Newfoundland, Canada. These infestations have resulted in extensive defoliation [1] causing up to 80% loss in incremental growth in balsam fir trees and recovery from this defoliation is slow [2].

In Newfoundland, the balsam fir sawfly has one generation per year. Eggs hatch in early June. Larvae are gregarious and feed openly on foliage that is one year old and older [3]. Pupation occurs in July and August followed shortly thereafter by adult emergence. Eggs are laid in current-year foliage where they overwinter [4].

The balsam fir sawfly nucleopolyhedrovirus (*Neab*NPV: Baculoviridae) was isolated from infected, field-collected balsam fir sawfly larvae from Newfoundland [5]. *Neab*NPV is being developed as a biological agent for balsam fir sawfly control. Here, we present results from *Neab*NPV field efficacy trials carried out in 2000 and 2001.

II. Materials and Methods

A. Aerial application of Neab*NPV*

In all aerial applications, *Neab*NPV, partially purified from infected balsam fir sawfly larvae cadavers, was applied in 2.5 L of 20% aqueous molasses/ha from Cessna 188 'Ag Truck' airplanes equipped with four, underwing Micronaire AU 4000 atomizers. *Neab*NPV was applied to three 50-ha balsam fir forest blocks (00-T1, 00-T2, 00-T3) located on the north side of Big Gull Pond on July 22 (2045 – 2130) and 23, (0559 – 0610) 2000 at a rate of 3 x 10^9 occlusion bodies (OBs)/ha. On July 21, 2001 (0615-0650), a 50-ha block (01-T1), located northwest of Stag Lake, was treated at a rate of 1x10^9 OBs/ha. In 2000, an untreated control block (00-C1) was a balsam fir stand of similar age and structure and was proximal to the 2000 treatment blocks. Treatment and control blocks were all located near Corner Brook, Newfoundland.

B. Assessment of Efficacy

To assess *Neab*NPV efficacy, 45-cm long, mid-crown balsam fir branch tips were taken from 30 trees along transects within each treatment and untreated control block immediately before *Neab*NPV application and each week for three weeks following the application. Balsam fir sawfly larvae on the treatment and control branches were counted and recorded.

C. Egg hatch and larval survivorship in 2001

Lower mid-crown branches with balsam fir sawfly eggs were collected from the 2000 treatment (00-T3) and untreated control (00-C1) blocks on June 22, 2001. The number of eggs per branch was determined and sufficient branches to yield approximately 300 eggs per block were selected. Needles near the cut end of each branch were removed and the end of each branch was re-cut, at an angle, under water to allow water-flow to the vessel elements of the branch. Immediately upon re-cutting, branches were transferred to either 450-mL Mason jars or 250-mL Erlenmeyer flasks, filled with 0.15% NaOCl-tap water, depending on the size of the branch. NaOCl was added to inhibit fungal and bacterial growth. The vessels containing branches were then placed in a north-facing room, at the Pasadena Field Station (Pasadena, Newfoundland), where

four large windows allowed indirect sunlight to penetrate the room. The temperature in the room ranged between 15 and 20°C. All branches were misted with tap water two to three times daily. Every fifth day, the NaOCl-tap water was changed and branch ends were re-cut as described above. Larvae were examined every two days beginning with first hatch through July 17, 2001 by which time, there were only two live larvae remaining on the branches from 00-T3. Dead larvae were individually placed in numbered 1.5-mL microcentrifuge tubes and were stored at -20°C for probing.

D. Cocoons from 2001

Balsam fir trees, within the 2001 treatment block (01-T1), were selected at random. Balsam fir sawfly larvae were collected *en masse*, 7-14 days post-spray, by beating each individual tree from mid- to lower-crown with a 2-m long wooden pole. All fallen debris was captured on a tarpaulin placed beneath the trees. The debris was poured into brown, paper bags (0.0255 m^3) independent of tree. Three, 30-cm branch tips of fresh foliage sprayed with 3 mL an aqueous suspension of *Neab*NPV ($1x10^7$ OBs/mL) were added to each bag. All bags were stapled shut and kept in the rearing room (as above) at the Pasadena Field Station. Larvae were reared in these bags until death or pupation. The mass collection bags were transported by truck to the Canadian Forest Service – Atlantic Forestry Centre (Fredericton, New Brunswick) on August 19-20, 2001 where, 50 non-melanized white and 50 brown melanized cocoons were selected for probing.

E. Neab*NPV molecular probing*

Nylon membranes (Pall and ICN© Biotrans) were pretreated by soaking in 10X SSC (1.5M NaCl; 0.15M $Na_3C_6H_5O_7{\cdot}2H_2O$, pH 7.0) for 5 minutes and dried on filter paper. Samples for probing were homogenized, in 1.5-mL microcentrifuge tubes, in an equal volume of water with sterile plastic pestles. A 3-µL aliquot of each homogenate was blotted onto the pretreated membranes and allowed to dry. The membranes were then treated to denature the nucleic acid contained in the samples by placing the membranes on filter paper saturated with denaturing solution (0.5M NaOH; 1.5M NaCl) at 65°C for 30 minutes then neutralized in 1.5M NaCl; 0.5M TRIS, pH 7.0 at 20°C for 1 minute.

NEN® Renaissance (NEN Life Sciences) detection protocol was followed for probing with minor deviations to reduce background noise and to increase sensitivity. Deviations included doubling the number of stringency washes and adding an extra rinse in 100mM NaCl; 50mM $MgCl_2$; 100mM TRIS, pH 9.5 for 15 minutes at 20°C before chemiluminescence detection.

The probe was made from an existing *Neab*NPV/EcoRI fragment library ranging in size from 3.5Kb to 5.5Kb. Briefly, *Neab*NPV DNA had been digested with EcoRI and the fragments were ligated into pT7/T3α-18 plasmid and amplified in *E.coli* DH5α-cells. Seven *Neab*NPV/EcoRI fragment (E, F, H, I, J, K, and L) plasmids were purified and digested with EcoRI to release the amplified inserts. The linearized plasmids and inserts were separated using gel electrophoresis. The inserts were excised and gel purified. The resultant fragments were pooled. NEN® Renaissance Random Primer Fluorescein Labeling Kit (NEN Life Sciences) was used to produce the probe by incorporating fluorescein-N^6-dATP to newly synthesized DNA using the *Neab*NPV/EcoR1 fragments as templates. The resultant probe consisted of labeled fragments, ranging from 300-600 bases, complementary to the target *Neab*NPV DNA. Biomax™ ML film (Eastman-Kodak) was used to record all results.

F. Statistical Methods

Neab*NPV efficacy in 2000 and egg hatch and larval survivorship in 2001* – The pre-spray populations in each 2000 treatment block and the control block were equated to 1 and the subsequent populations in each respective block were expressed as a proportion of the pre-spray population. The number of live larvae per egg was natural log transformed and regressed against time as a second-order polynomial function for the spray and control sites (REG/SAS release 8.02). The effect of site on the probability of larval infection through time was determined using binary logistic regression (GENMOD/SAS release 8.02).

Cocoons from 2001 - Binary logistic regression was utilized to model the probability of *Neab*NPV infection in cocoons as a function of cocoon colour. (GENMOD/SAS release 8.02).

III. Results

A. Neab*NPV efficacy in 2000*

Two weeks after *Neab*NPV was applied, larval population densities in one treatment block and in the control were similar to those just before the spray (Fig. 1). However, larval densities in the other two treatment blocks had declined by approximately 50% two weeks after the spray and larval populations in all three treatment blocks had declined to a much greater extent than that observed in the control block three weeks after *Neab*NPV application (Fig. 1).

B. Egg hatch and larval survivorship in 2001

The percentage of successful hatch in 2001 was lower for eggs on balsam fir branches collected from the treatment block compared to those collected from the untreated control block (Table I) resulting in fewer larvae on the *Neab*NPV-treated foliage at the beginning of laboratory rearing (Table I, Fig. 2). Larval mortality began earlier and reached a higher incidence in larvae from the *Neab*NPV-treated than control foliage (Table I, Fig. 2). Probing of dead larvae showed that there was a higher percentage of *Neab*NPV infection in

larvae from treatment than control blocks (96.2% versus 81.1%). Logistic regression also indicated that the probability of a larva being infected with *Neab*NPV was significantly dependent on whether it came from the treatment or control block ($X^2_1 = 6.68$, p=0.0097).

C. *Cocoons from 2001*

Probing results showed that white cocoons had a higher *Neab*NPV prevalence (58%) compared to brown cocoons (14%) and that cocoon colour (white versus brown) had a significant association with cocoons testing positive for *Neab*NPV ($X^2_1 = 15.66$, $p < 0.0001$).

TABLE I

Percent egg hatch and larval mortality on balsam fir branches collected in 2001 from year 2000 *Neab*NPV-treated (00-T3) and untreated control (00-C1) blocks. Sample sizes are indicated in parentheses.

Treatment	Egg Hatch (%)	Larval Mortality (%)
*Neab*NPV	65.7 (302)	44.0 (111)
Control	94.4 (285)	31.8 (183)

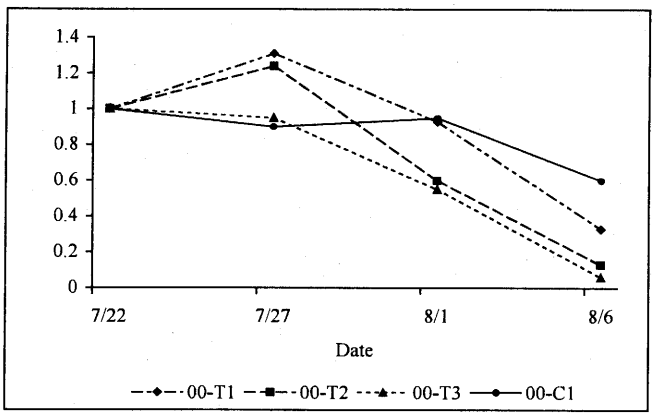


Fig. 1. Survival of balsam fir sawfly larvae in three *Neab*NPV treatment blocks (00-T1, 00-T2, 00-T3) and one untreated control (00-C1) as a proportion of their pre-spray (sampled July 22, 2000) population levels.

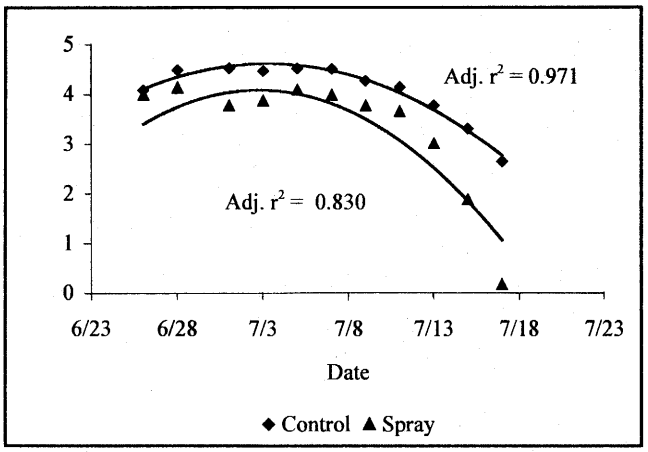


Fig. 2. Second-order polynomial regression for the natural log transformed number of larvae per egg through time for larvae on foliage from the year 2000 *Neab*NPV-treated (00-T3) and untreated control (00-C1) blocks in 2001 ($F_{2,19}$=52.34, p<0.0001 and $F_{2,8}$=167.60, p<0.0001, respectively).

IV. Discussion

Population crashes, due to NPV epidemics, occur in many species of sawflies [6]. NPV transmission is density-dependent and sawflies are particularly susceptible to the communication of NPVs because many are communal and feed openly on foliage [7]. Sawfly NPVs only infect the midgut epithelium so that following a single replicative cycle, infected cells containing OBs, are sloughed off into the frass and out of the body where the OBs can infect other hosts [8]. For these reasons, attempts to use NPVs to suppress or eliminate sawfly populations have usually met with success [6, 7]. Balsam fir sawfly larvae are gregarious, open feeders on balsam fir foliage and are subject to *Neab*NPV epizootics. This study indicates that application of *Neab*NPV can suppress balsam fir sawfly populations to tolerable levels by causing high mortality in both the year of the application and during the subsequent year.

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