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**Report Title**: Balsam Fir Sawfly (Neodiprion Abietis) Nucleopolyhedrovirus: Field Efficacy Trials

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# *The Fundy Model Forest…*
# *…Partners in Sustainability*

*"The Fundy Model Forest (FMF) is a partnership of 38 organizations that are promoting sustainable forest management practices in the Acadian Forest region."*

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**Final Report to the Fundy Model Forest 2002-2003.**

**Balsam Fir Sawfly (*Neodiprion abietis*) Nucleopolyhedrovirus: Field Efficacy Trials**

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*Introduction* - Widespread infestations of balsam fir sawfly (*Neodiprion abietis*) in western Newfoundland threaten substantial investments in silviculture and the long-term wood supply of the forest industry in this province. Options to control balsam fir sawflies are few but, in 1997, we isolated a nucleopolyhedrovirus (NPV) from larvae of the balsam fir sawfly (hence NeabNPV) from western Newfoundland. Since then, we have been working to develop and register NeabNPV as a biological control agent against this forest insect pest.

To register a biological control product with the Pest Management Regulatory Agency (PMRA) for operational use, it is necessary to prove that the product is effective and safe both environmentally and for public health. Here, we report on our progress to meet the requirements of PMRA for the registration of NeabNPV.

*Field trials* **-** On July 22-23, 2000, three blocks, each 50 ha in area, between Pinchgut Lake and Big Gull Pond near Corner Brook, NF, were treated aerially with NeabNPV at 3 x $10^9$ OBs/ha. (In all trials, a 20% aqueous solution of molasses was used as the carrier for the virus and the mixture was applied at a rate of 2.5 L/ha using Cessna 188 AgTrucks equipped with Micronaire AU 4000 atomizers). Aerial field trails (1 x $10^9$ OBs/ha) were conducted on July 21-22, 2001, east and north of Stag Lake near Corner Brook and north of St. Alban's, Bay D'Espoir, on July 24, 2001. The three blocks near Stag Lake totaled 2200 ha and the block in the Bay D'Espoir was 600 ha. On July 21-23, 2002, a total of approximately 5000 ha was treated (1 x $10^9$ OBs/ha) in three blocks to the south, east and north of Corner Brook. In all trials, in all three years, there was good deposit on the targeted areas resulting in significantly higher larval mortalities in the spray blocks compared to the control blocks. Additionally, it was found that the number of balsam fir sawfly pupae and eggs was lower in the spray blocks compared to the control blocks. In the years immediately following NeabNPV applications, we have found that the number of eggs per shoot, the percentage of successful egg hatch and the resultant number of larvae per shoot were lower in the spray blocks than in the controls. As a result, defoliation in the control blocks was much greater than in any of the spray blocks, which had little defoliation.

The results of the aerial application trials have demonstrated that i) NeabNPV is easy and cheap to produce, ii) our formulation allows for smooth flow from the aircraft and good deposit on the foliage, iii) a single application at 1- 3 x $10^9$ OBs/ha against second instar larvae results in large reductions in the larval population within 15 days and iv) applications in one year adversely affect the population of balsam fir sawfly larvae in the following year resulting in significantly decreased defoliation.

*Silvicultural practices and sawfly pests* – G. Moreau (Ph.D. candidate UNB) has shown that BFS population cycles are of equal length in PCT and unthinned balsam fir stands. However, BFS

populations reach higher densities and cause more defoliation in PCT than unthinned stands. In both instances the principle cause of the population crash is NeabNPV.

*NeabNPV balsam fir sawfly bioassays:* Second, third and fourth instar balsam fir larvae were challenged with NeabNPV using imbibing assays by J. Dedes and K. van Frankenhuyzen (Canadian Forest Service – Great Lakes Forestry Centre, Sault Ste. Marie, ON). The $LD_{50}$ at 7 days post-inoculation (95% confidence limits) for second instars was 87.4 OBs (7.1-304.9) and 2074.8 OBs (813.1-3999.0) for third instars. $LD_{50}$s at 7 days post-inoculation could not be determined for fourth instars because both the treated and control larvae died of apparent natural infection by NeabNPV before the conclusion of the assay.

*NeabNPV non-target invertebrate testing:* NeabNPV assays against the following insect species have been carried out with the collaboration of K. Barber and S. Holmes (Canadian Forest Service – Great Lakes Forestry Centre, Sault Ste. Marie, ON): *Acantholyda erythrocephala* (pine false webworm), *Diprion similis* (introduced pine sawfly), *Gilpinia hercyniae* (European spruce sawfly), *Pristiphora geniculata* (Mountain ash sawfly) (Hymenoptera: Diprionidae), *Megachile rotundata* (alfalfa leafcutter bee) (Hymenoptera: Megachilidae) and *Clepsis persicana* (white triangle leafroller) (Lepidoptera: Tortricidae). Additionally, we challenged *Apis mellifera* (honey bee) (Hymenoptera: Apidae) in hives with NeabNPV. Exposure to NeabNPV did not cause mortality significantly different from controls in *C. persicana*, *M. rotundata* or *A. mellifera*. In sawfly species, *A. erythrocephala*, *D. similis, G. hercyniae* and *P. geniculata*, there was significant mortality associated with treatment with NeabNPV compared to controls. Affected nontarget sawfly larvae were all probed with NeabNPV genomic DNA probes and all were negative for NeabNPV. These results are very interesting, considering that all previous reports have indicated sawfly NPVs to be highly host specific. We are continuing to investigate the cause of the nontarget sawfly mortality. B. Whittome (Ph.D. candidate University of Victoria) is studying NeabNPV infection in insect cell cultures (*Choristoneura fumiferana* – Cf70; *Neodiprion lecontei* – NL-10, NL-18, NL-28). A post-doctoral fellow (PDF - Dr. E. Becker, UVic), funded by the Biocontrol Network, is looking at *in vivo* and *in vitro* NeabNPV infection processes.

*NeabNPV functional genomics:* Restriction analyses for NeabNPV have been done and the genome size has been determined to be 95 Kb. The genome of NeabNPV has been fragmented and cloned into sequencing vectors. Comparisons between completed sequence fragments of the NeabNPV genome and known baculovirus sequences have identified a number of putative baculovirus genes and ORFs.

*NeabNPV vertebrate toxicity and infectivity* – Many of the requirements for PMRA registration have been completed. Requirements for environmental toxicology and human health are yet to be done. These tests involve trials using non-target vertebrates, including fish, birds and mammals. Due to licencing and permit requirements need to carry out these tests, we will have to contract the work out. BC Research and Alberta Research have been approached to carry out these tests. Additionally, mammalian cell line toxicity and infectivity tests will also have to be done. These tests have been intitiated by B. Whittome and NeabNPV does not appear to cause any cytopathic effects to several cell lines: Hela (human cervical cancer), HEK-293 (human

embryonic kidney), HT-1080 (human fibral sarcoma), NIH-3T3 (Swiss albino mouse). Further trials assessing for viral DNA replication and cellular antigenic responses are underway.

*NeabNPV shelf-life bioassays* – The PMRA requires that microbials be assessed for storage shelf-life. Basically, NeabNPV will have to be stored at a range of temperatures and assessed, by host bioassays, at set intervals, for example 6, 12, 18, 24 months. It is possible that we will have to run parallel bioassays of two NeabNPV treatments. In our NeabNPV production – purification process, we are not able to eliminate all bacteria. If any of these bacteria prove to be potential human pathogens, PMRA requires that the batch be destroyed. We have found that if we pasturize the NeabNPV suspension at 55°C for 15 minutes, we can reduce the bacterial counts a thousand-fold. In initial bioassays, "pasturized" NeabNPV remains active. In other NPVs, pasturization does not affect viral efficacy in the first year but, it does reduce potency in subsequent years (C. Ignoffo, USDA, Columbia, MO, pers. comm.). Pasturization might only be necessary to "save" a contaminated batch but, if it is included in the production process, its effect on shelf-life will have to be assessed.

*Benefits and deliverables of NeabNPV research*

- A registered NeabNPV product for operational use against BFS and other sawfly species.
- Template for PMRA requirements for baculovirus registration, including a list of commercial facilities competent to carry out vertebrate toxicology and infectivity tests.
- Strategies for efficacious NeabNPV application in integrated pest management programs.
- Epidemiology and impact of NeabNPV on natural populations of balsam fir sawfly.
- Contribution to our knowledge and understanding of baculovirus evolution and phylogeny.