

Registration Pursuant of Section 7 of the Environmental Assessment Act

Project Title

**Experimental Application of the Balsam Fir Sawfly
Nuclear Polyhedrosis Virus (NPV)
Against Its Natural Host, the Balsam Fir Sawfly**

May 2002

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Experimental Application of the Balsam Fir Sawfly Nucleopolyhedrovirus Against Its Natural Host, the Balsam Fir Sawfly

Nature of Proposed Pesticide Application

The Province of Newfoundland and Labrador continues to face serious and widespread infestations of balsam fir sawfly (*Neodiprion abietis*). These infestations are threatening the substantial investment in silviculture and consequently the long-term wood supply for the forest industry. For a third year, the Canadian Forest Service (CFS), in co-operation with the Newfoundland Department of Forest Resources and Agrifoods (DFRA) and Forest Protection Limited, is proposing to carry out an experimental research application of a highly species-specific microbial biological control agent (balsam fir sawfly nucleopolyhedrovirus–NeabNPV) on selected silviculturally treated forest stands that are forecast to receive moderate to severe the balsam fir sawfly defoliation in 2002 and that are at the leading edge of the infestation. Applications of this biological control agent will be made using fixed-wing aircraft.

Description of Balsam Fir Sawfly Problem

Insect Population Levels

The balsam fir sawfly (BFS) is native to and has been an occasional pest on balsam fir in Newfoundland. Since the early 1990s, it has become more important as a pest of young and semi-mature balsam fir, particularly in pre-commercially thinned stands (PCTs). The population overwinters in the egg stage in fir needles, and larvae usually hatch in late-June to mid-July, depending on the weather. Larvae feed on previous-year and older foliage for a number of weeks before pupating. Adult sawflies emerge in August, mate and lay eggs in the needles of the current year. Populations have been regulated by natural diseases, parasites and predators. Outbreaks have normally been of short duration (3 to 4 years) and were terminated by natural factors, predominantly NeabNPV. Although localized damage was often severe, tree mortality has been limited. Defoliation, however, can cause significant growth loss to affected trees without tree death. Research at CFS has shown that, after defoliation has ceased, there may be a 13 to 18 year period of reduced growth before the trees recover to pre-infestation growth rates (Piene et al. 2001).

The current BFS infestation in western Newfoundland was detected in 1991 near Bottom Brook, east of Stephenville. By 1994, approximately 1216 hectares (ha) of defoliation were recorded. In 1995, moderate and severe defoliation was mapped on 12,600 ha, with some 10% mortality occurring in young fir stands. The infestation continued to expand in 1996 with defoliation on 19,700 ha, including 15,400 ha in the moderate and severe categories. In 1997, the infestation expanded to the northeast and southeast into larger areas of valuable PCT balsam fir stands. In 1997, 53,000 ha were defoliated, with 30,300 ha in the moderate and severe categories. Pockets of defoliation were also detected on the Burin Peninsula and in Bay d'Espoir. The moderate and severe defoliation in 1998 totalled approximately 24,400 ha, with 16,500 ha occurring in western Newfoundland, 5800 ha in Bay d'Espoir and 2100 ha on the Burin Peninsula. In 1999, moderate and severe defoliation occurred on 18,400 ha, with 12,400 ha in western Newfoundland, 3300 ha in Bay d'Espoir and 2800 ha on the Burin Peninsula. In

2000, approximately 22,000 ha in western Newfoundland and 19,000 ha in the Bay d'Espoir were defoliated. Moderate to severe defoliation was recorded on 38,000 ha in western Newfoundland and 9000 ha in the Bay d'Espoir in 2001.

Forecast for 2002

The moderate to severe defoliation forecast for 2002 is for 57,400 ha in western Newfoundland. This area extends from south of Grand Lake north to Old Man's Pond and from Stag Lake–Cooks Brook across the Humber Arm near Gillams and east to Steady Brook–Corner Brook Lake. This area is of particular concern because its significant proportion of PCT stands, which have been established at an average cost of \$1000+/ha (a total amount in excess of \$10 million). These stands are critical to maintaining an adequate wood supply for the forest industry. The impact of balsam fir sawfly infestations, if left unchecked, will result in substantial loss of this investment. The failure to adequately protect the investment in silviculture, and the potential loss of future harvestable stands, would be significant to the social and economic well-being of the people, particularly on the west and southwest coasts of the Island. This is true both in terms of direct employment and in spin-off economics.

Apart from NeabNPV, there does not appear to be any other significant natural factor influencing BFS populations. With prolonged, severe defoliation, affected trees will be stressed, lose growth and be subject to mortality from secondary insects and diseases. Since the BFS outbreak began, it is estimated that the Province has lost in excess of 2 m³ of growth per hectare per year, that is, a loss of more than 120,000 m³ of incremental growth.

Control Options

A pest management program is being developed against the BFS in Newfoundland to protect valuable young stands and silviculturally treated areas of balsam fir. The purpose of the program is to reduce BFS population levels in treated areas to minimize the loss of foliage and tree growth and to prevent tree mortality due to secondary infestations in trees weakened by BFS attack. Unfortunately, control options for BFS are limited. Experimental programs were carried out by CFS and its collaborators in 1998, 1999, 2000 and 2001 in Newfoundland and in other jurisdictions to develop biological control options for a number of sawflies, including the BFS and the yellowheaded spruce sawfly. Progress has been made and work is continuing. Four control options (chemical, biological, botanical and viral) are described below.

Dylox

The organophosphate insecticide Dylox 420 (trichlorfon) was used in 1998 under an emergency registration from the Pest Management Regulatory Agency (PMRA). The results of experimental trials conducted in the same year showed that lower dosages than those recommended could be effective. DFRA requested registration of Dylox and, for 1999 only, PMRA granted a temporary registration for Dylox for use against the BFS. Dylox is fully registered for use against the yellowheaded spruce sawfly but is no longer being pursued further for BFS because of public resistance to its use and issues surrounding buffer zones. In 1998, buffer zones for Dylox around water bodies were established at 100 m at the federal level and 200 m at the provincial level. In 1999, buffer zones were established at 200 m both

federally and provincially. This resulted in the further exclusion of significant areas of infested stands from the protection program.

Bacillus thuringiensis

The most common biological insecticide to be applied aerially in forests against the spruce budworm and hemlock looper is *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*). *B.t.k.* was developed as a control product for certain pest insects belonging to the order Lepidoptera (butterflies and moths). To be effective, *B.t.k.* must be ingested by an appropriate host insect. A protein crystal within the wall of the bacterial spore must first be digested by specific proteases within the alkaline midgut of the host insect. The *B.t.k.* toxin must bind to specific receptors on the midgut epithelial cells to work. However, sawflies belong to the order Hymenoptera (bees, ants and wasps), and their larvae are not susceptible to *B.t.k.*

Bacillus thuringiensis var. *israelensis* (*B.t.i.*) is registered for use in the control of mosquito and blackfly (order Diptera) larvae. Its mode of operation is the same as that of *B.t.k.* but proteins digested from the larger crystal bind specifically to receptors on the cells of the midgut of the insects. In 1999, *B.t.i.* was tested experimentally against BFS on a small area; *B.t.i.* was found to be ineffective.

Neem

Neem (azadirachtin) is a botanical insecticide extracted from the neem tree (*Azadirachta indica*) native to India and parts of Africa. Certis (a manufacturer of one neem product) applied for and received temporary registration from PMRA for Neemix 4.5 for use against several sawfly species including the BFS. Neem has a number of properties that affect target pests. Depending on the amounts applied, these include insecticidal, insect growth regulatory and anti-feedant activities. Neem is registered for use in many countries including the USA where it is registered for indoor and outdoor use. It can be applied from the air and ground to horticultural-ornamental plants, trees, shrubs and agricultural crops. An operational program using Neemix 4.5 was carried out by DFRA on about 1500 ha in the Bay d'Espoir. Problems were encountered with the formulation of this product, which the manufacturer will have to resolve these before any future applications.

BFS nucleopolyhedrovirus

Nucleopolyhedroviruses (NPVs) are found only in arthropods, primarily insects. NPVs have a high degree of host specificity affecting a single insect species or only ones that are closely related. NPVs are not related to any known human, veterinary or plant pathogenic viruses. Specificity and safety tests of NPVs have shown that there are no toxicological or other deleterious effects to mammals, birds, amphibians, aquatic microorganisms, beneficial and other nontarget insects. Population crashes because of NPV epidemics occur in many species of sawflies (Hymenoptera: Diprionidae) (Table 1). Here, NPV infection is density dependent, and these insects are particularly susceptible to the communication of disease as most are communal and feed openly on foliage. NPVs are transmitted through ingestion by a suitable host larva. Viral occlusion bodies (OBs) dissolve in the midgut, releasing the virions to infect midgut epithelial cells. 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 they can infect other host insects. Death normally occurs within one to two

weeks; during that time, however, the host is producing infective units of the disease. Sawfly NPVs are highly host specific, and it has been necessary to develop a different virus for each host species. Attempts to use NPVs to suppress sawfly populations have usually met with success (Table 1).

Table 1. Application and efficacy of nuclear polyhedrosis viruses against sawfly larvae.

Diprionidae	Natural Habitat	Aerial Spray	Epizootics OBs/ha	Control
European pine sawfly <i>Neodiprion sertifer</i>	open communal	yes	5.1×10^{10} to 3.9×10^{11}	yes
Redheaded pine sawfly <i>N. lecontei</i>	open communal	yes	1.3×10^9 to 5.5×10^9	yes
Swaine jack pine sawfly <i>N. swainei</i>	open communal	yes	9.4×10^9 to 7.5×10^{10}	yes
Red pine sawfly <i>N. nanulus</i>	open communal	yes	ground only	yes
Jack pine sawfly <i>N. pratti banksianae</i>	open communal	yes	ground only	yes
Balsam fir sawfly <i>N. abietis</i>	open communal	yes	1.0×10^9 to 3.0×10^9	yes
European spruce sawfly <i>Diprion hercyniae</i>	open solitary	yes	ground only	yes
Redheaded jack pine sawfly <i>N. rugifrons</i>	open communal	yes	ground only	no

Progress of NeabNPV Research 1997–2001

Field trials

In 1997, CFS researchers isolated an NPV from larvae of the BFS (*Neodiprion abietis*, hence NeabNPV) from western Newfoundland. Since then, they have been working to develop and register NeabNPV as a biological control agent against this forest insect pest. In 1999, NeabNPV was applied to 1 ha of balsam fir forest to field amplify the virus. From this 1-ha application, enough NeabNPV was obtained to treat 1800 ha of forest at an application rate of 1×10^9 OBs/ha. In 2000, three 50-ha blocks were treated aerially with NeabNPV at 3×10^9 OBs/ha (Tables 2–4). 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 a Cessna 188 AgTruck equipped with Micronaire AU 4000 atomizers. Results from all NeabNPV spray blocks showed decreased BFS larval survivorship compared to the controls (Table 4).

In 2001, CFS researchers continued to monitor the year 2000 spray blocks and controls. (A second unsprayed control block was added for monitoring in 2001.) Each block and control was sampled once a week beginning on June 3 and ending on July 19, 2001. Egg and larva counts were made from 30 trees per block (three trees per plot from 10 plots per spray or control block). 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 they were in either of the two controls (Fig. 1). As result, defoliation in the control blocks was much greater than in any of the spray blocks where there was little defoliation.

Aerial field trails were conducted on July 21 and 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 comprised 2200 ha and the Bay D'Espoir block 600 ha. NeabNPV was applied to an area approximately equivalent to 713 ha within these four blocks (Table 5). NeabNPV was applied at a rate of 1×10^9 OBs/ha in 2.5 L/ha 20% aqueous molasses using two Cessna 188 AgTrucks equipped with Micronaire AU 4000 atomizers. (Aircraft were provided by Forest Protection Limited.) There was good deposit on the targeted areas and there were higher larval mortalities in the spray blocks compared to the control block. It was also found that the number of BFS pupae and eggs was lower in spray blocks NFLD 1 and 3 compared to the control block (Fig. 2).

Results

In the 2000 and 2001 NeabNPV field trials, CFS scientists found 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 $1-3 \times 10^9$ OBs/ha against second instar larvae results in large reductions in the larval population within 15 days and
- iv) application in one year appears to affect the population of BFS larvae in the next year resulting in significantly decreased defoliation.

Table 2. Aerial spray application parameters for NeabNPV, Newfoundland, 2000.

Parameter	Block		
	NeabNPV-C	NeabNPV-B	NeabNPV-A
Aircraft	Cessna 188 C-GMZJ		
Date	July 22	July 22	July 23
Start Times	20:45	21:20	05:59
Finish Times	21:18	21:30	06:10
Track spacing	23 m		
Atomizers	4 x Micronair AU 4000		
Track flow rate	17.5 L/min	20 L/min	20 L/min
Average flow rate	21.5 L/min	20.0 L/min	20.4 L/min
Target Application rate	2.50 L/ha	3.0 L/ha	3.0 L/ha
Average application rate	3.32 L/ha	3.19 L/ha	3.10 L/ha
Total spray line length	20,374 m	7294 m	7558 m
Total area sprayed	46.9 ha	16.9 ha	17.4 ha
Air temperature: Start	15°C	14°C	14°C
Finish	14°C	14°C	13°C
Relative humidity	93%–99%	90%–87%	95%–92%

Table 3. Summary of NeabNPV spray deposit on Kromekote cards, Newfoundland, 2000.

Spray Block	Collection Date	N	Spray Droplets/cm ²
NeabNPV-C	July 22, pm	10	26.98 ± 15.18
NeabNPV-B	July 22, pm	5	10.04 ± 8.62
NeabNPV-B	July 23, am	10	8.54 ± 5.92
NeabNPV-A	July 23, am	10	3.04 ± 2.87

Table 4. Summary of balsam fir sawfly population assessment of living larvae per branch in NeabNPV treatment and non-treatment control plots, Newfoundland, 2000.

Date	Living Larvae/Branch			
	Neab NPV-A	Neab NPV-B	Neab NPV-C	Control
July 22	20.4	43.4	30.9	34.5
July 27	26.8	53.7	26.4	31.1
August 1	18.9	26.1	17.0	32.8
August 6	6.8	5.8	2.0	20.8
% Survival	33.4	13.3	6.5	60.3

Table 5. Aerial spray application parameters for NeabNPV, Newfoundland, 2001.

Parameter	Nfld1.no1	Nfld2.no1	Nfld3.no1	Sb211.no1
Product	NeabNPV	NeabNPV	NeabNPV	NeabNPV
Aircraft type	2-Cessna 188	2-Cessna 188	2-Cessna 188	2-Cessna 188
Aircraft speed	177 km/hr	177 km/hr	177 km/hr	177 km/hr
Date	July 22, 2001	July 21, 2001	July 21, 2001	July 24, 2001
Start Times	06:00	09:15	06:15	11:56
Finish Times	07:45	10:15	06:50	12:33
Track spacing	25 m	25 m	25 m	25 m
Atomizers - No., type	4 x Micronair AU4000	4 x Micronair AU4000	4 x Micronair AU4000	4 x Micronair AU4000
Flow rate	18.4 L/min	18.4 L/min	18.4 L/min	18.4 L/min
Average flow rate	18.4 L/min	18.4 L/min	18.4 L/min	18.4 L/min
Target application rate	2.5 L/ha	2.5 L/ha	2.5 L/ha	2.5 L/ha
Average application rate	2.5 L/ha	2.5 L/ha	2.5 L/ha	2.5 L/ha
Total area sprayed	328 ha	335 ha	50 ha	108 ha

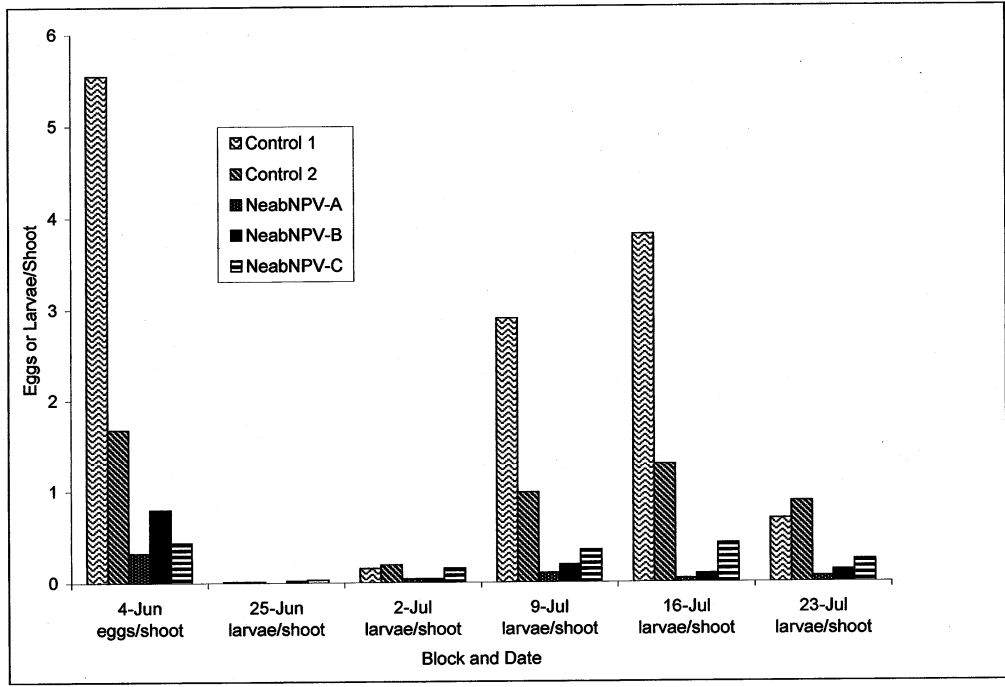


Figure 1. Egg and larval densities in control and spray 2000 blocks in 2001.

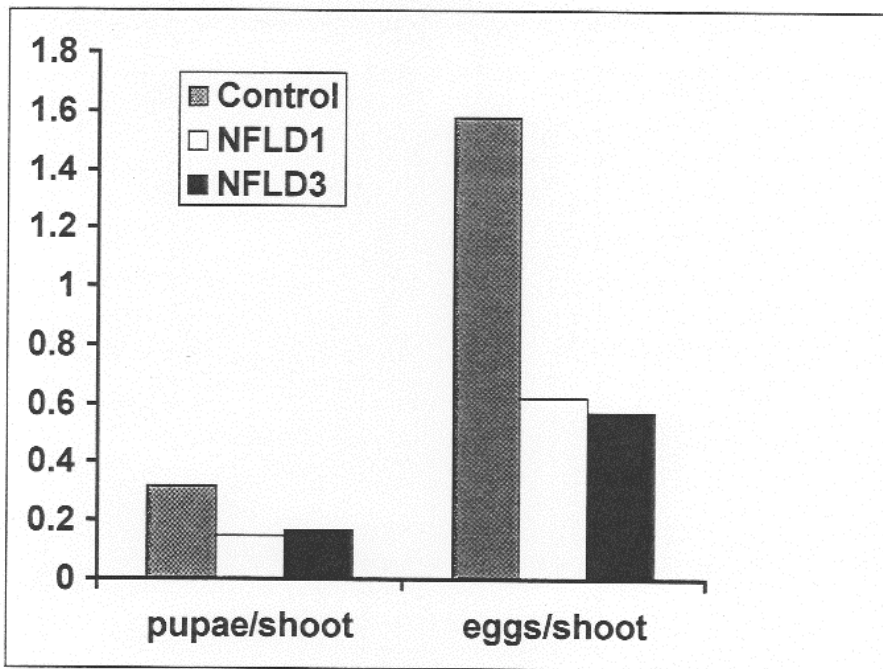


Figure 2. The number of balsam fir sawfly pupae and eggs on balsam fir shoots on trees in the spray 2001 blocks NFLD 1 and NFLD 3 compared to the control block.

Spray Procedures

Since 1977, DFRA has assumed responsibility for all operational control programs conducted against forest insect and disease pests. However, for the NeabNPV research trial applications, CFS has taken the lead in cooperation with DFRA. CFS staff researchers have many years of experience with experimental aerial trials of new biological control agents and in operational control programs that use registered control products. The insect population forecast carried out by DFRA staff will be used to predict infestation levels for summer 2002 and to identify proposed treatment areas. CFS staff will carry out other aspects of the NeabNPV research aerial program, including the transportation, handling, mixing and loading of the trial product onto the aircraft. CFS and DFRA staff will oversee the actual aerial spray to ensure that the proper areas are treated under the appropriate weather conditions and that all Licence stipulations are followed. CFS staff will monitor insect and host-tree shoot development and larval numbers throughout the season. They will also determine the application date(s) and priorities of areas to be treated. Monitoring to determine NeabNPV efficacy will continue for several weeks following the actual spray. Assessments will continue to be made following larval feeding, pupation, emergence of the adults and oviposition of the eggs. All necessary ground, communication and sampling equipment is owned and will be supplied by CFS; CFS and its co-operators use appropriate current equipment and technology.

CFS complies with existing regulatory guidelines. Navigation of spray aircraft is by a Differential Global Positioning System (DGPS) located inside the aircraft. Parameters of the spray blocks will be determined on the ground with handheld GPS personal navigators; these coordinates will be transferred to the onboard aircraft computer. At the time of the spray application, a supervisor in an accompanying aircraft will observe the application from above to determine the accuracy and performance of the spray aircraft and will initiate corrective action as necessary. CFS supervisors will assess the favourability of weather parameters before and during spray application. To ensure environmental safety, spray bases will have appropriate, current and approved safety and emergency equipment, materials and methods.

Worker Safety

CFS has well-established safety guidelines for workers involved in insect control. Personnel handling the NeabNPV formulation will wear the required safety equipment as indicated on the experimental label during mixing and loading onto the aircraft. In addition, CFS will follow all approved safety precautions and established rules and guidelines concerning personal hygiene of all mixer/loader personnel working with NeabNPV formulations, as indicated on the experimental label. Hospital and emergency telephone numbers will be posted in a conspicuous place to be used in the event of accident. Applicable contingency measures will be available to personnel in the event of an accident.

Public Health Considerations

To minimize the risk of exposure of people to insecticide spray, “no-spray” buffer zones will be left around known places of permanent human habitation and around areas such as cabin developments, parks, camps and day-use areas. In 2002, spraying near habitation will be subject to terms and conditions of the Operator’s Licence from the Department of Environment and Labour in consultation with the appropriate Health and Community Services personnel. Cabins will be adequately buffered in relation to the product being applied. In

addition, a 1.6-km buffer zone will be left around identifiable intakes to known community water supplies. If, during the course of a spray mission, unauthorized personnel are detected in or near a treatment area, the aerial supervisor will instruct the spray aircraft pilot to provide extra buffers or to terminate the mission, as circumstances dictate.

Environmental Safety

In terms of environmental safety, all stipulations in the licence issued by the provincial Department of Environment and Labour will be followed. These include the reporting of any incidents, such as spills, to the appropriate authorities. CFS and DFRA have contingency plans that are reviewed and approved annually prior to receiving an Operator's Licence. These plans outline procedures for spill reporting, emergency first aid for exposure, aircraft crash in bush, aircraft accident on or near the airport, jettisoned aircraft load, drum decontamination and disposal and other general regulations and instructions as necessary.

Public Notification

As part of the program, the public and media in the vicinity of the proposed treatment areas will be notified prior to commencement of the program through advertisements and news releases and through appropriate direct contact if required. Information will include product details, spray block locations, timing of application and contact numbers. Access roads to the spray areas will be posted with signs indicating treatment, product, dates and phone numbers for more information. A phone-in information line will be set up so that people can call to find out the status of areas receiving treatment.

Regional offices of DFRA and the Department of Environment and Labour, as applicable, will be provided with maps showing spray blocks, which will be available for public viewing during regular office hours. District offices of the DFRA will be made aware of spray blocks in their area and provided with applicable detailed maps so they can inform the public on specific local blocks when requested. Also, Dr. Christopher Lucarotti (CFS-AFC), who is in charge of the efforts to register NeabNPV for operational use against the BFS, will be present in Newfoundland during the spray period and at least one week before and two weeks after the spray. He may be called upon at other times to assist in answering questions and concerns from the public.

Potential Spray Conflicts

There are always potential conflicts with insect control programs, such as proximity to habitation and water supply and recreational (fishing, camping, berry picking) areas and potential impacts on wildlife. However, in approving a product at the federal registration level and in granting a licence at the provincial level, mitigating measures are identified that can eliminate or significantly reduce the potential for conflicts. These mitigating measures are outlined on the product label as approved by the PMRA and in terms of any buffer zones as stipulated in the Operator's Licence. In addition, the proponent is also required to post signs and advise the public about the program to lessen accidental exposure.

Integrated Pest Management Approach

In 1997, a cooperative research agreement involving the CFS, DFRA, Corner Brook Pulp and Paper Ltd. and Abitibi-Consolidated Inc. was initiated to investigate BFS ecology. The prevalence of natural control factors such as viruses, fungi and parasites and their effect on

BFS populations are under investigation. The impact of the BFS on and differences observed between thinned and unthinned stands is also under investigation. In 1998, additional financial resources were obtained through a Natural Sciences and Engineering Research Council (NSERC)–CFS–Industry grant administered through the University of New Brunswick, with funding continued through 2001. Funding for 2000–2003 was also obtained from the CFS Biotechnology Strategy by CFS researchers to study the functional genomics of NeabNPV. Additional funds have been obtained from the NSERC BioControl Network for the period 2001–2006. We hope that these cooperative research programs, by identifying natural factors that influence BFS populations, will lead to an integrated pest management strategy against this pest.

In November 2000, CFS research staff consulted with PMRA officials on the pre-submission of the registration. The purpose of the consultation was to determine the requirements necessary to register NeabNPV for operational use. Progress has been made in this direction over the last four years, including i) two years of field efficacy trials, ii) four years of field work by three graduate students studying BFS ecology, iii) the full sequencing of the NeabNPV genome and iv) the completion of bioassays against non-target insects. We hope that an application for NeabNPV registration will be submitted to PMRA no later than spring 2003.

Approval Process

Any chemical manufacturer who wishes to sell a pesticide in Canada must first register that pesticide under the Pest Control Products Act. To receive registration, the manufacturer must follow the registration process administered by PMRA. Registration involves the submission of an application by the manufacturer. The company must first carry out extensive studies on the product. The application must be supported by a very thorough data package documenting the effects of the pesticide on users, bystanders and the environment

PMRA then performs a scientific evaluation of the product. This evaluation may take years, as it may require the study of long- and short-term human health effects, residues in food, ground water contamination, effects on wildlife and environmental fate. A registration will be granted only if the safety of the pesticide and its merit and value for the proposed use are found to be acceptable. If problems with the product are identified, registration will not be granted. All products are subject to re-evaluation, with provision for suspension or cancellation.

Once the federal government approves a registration, the provincial governments become more involved. Each province has legislation dealing specifically with pesticide use in that province. In Newfoundland and Labrador, pesticide use is regulated under the Pesticides Control Act. This legislation requires all organizations and companies using pesticides to apply for and receive a Pesticide Operator Licence. This licence regulates aspects of an operation not covered by federal legislation and requirements. As with federal regulations, the Pesticide Operator Licence is designed to minimize risk to human health and the environment. Aspects of a pesticide operation such as buffer zones, spill response, public information and notification programs, monitoring requirements and weather conditions are all specified in the licence as they relate to a particular spray program. The federal registration system, combined

with the provincial licensing and regulatory system, ensures that any pesticide that is used in Canada has passed a comprehensive environment/health evaluation.

Provincial legislation also requires individuals to be trained in the safe use of pesticides. Only individuals that successfully pass the provincial pesticide applicator exam (administered by the Pesticides Control Section of the Department of Environment and Labour) are granted an applicator licence and authorized to handle pesticides. Compliance and enforcement activities are also carried out by the Pesticides Control Section.

As with all commercial pesticide operations, the 2002 experimental NeabNPV program will be regulated by the Pesticides Control Section of the Newfoundland Department of Environment and Labour.

Schedule

We wish to make an aerial application of NeabNPV against first instar BFS larvae early in July 2002. In 2000 and 2001, NeabNPV was applied against second instar BFS larvae. As a result, larvae were able to feed and cause significant defoliation before succumbing to the virus. We believe that an application of NeabNPV at the 2001 rate (1×10^9 OBs/ha in 2.5 L/ha 20% aqueous molasses) would kill first instar BFS larvae with the same residual effects seen in the 2000 spray one year later and would provide foliar protection in the year of application. In the two previous trials, no adverse effects to the environment or to human health have been observed by us and, to our knowledge, none have been reported by any other party. Proposed sites of aerial application of NeabNPV include block 201 near Stag Lake (Fig. 3). The infestation has now crossed the Humber Arm, and we propose to spray this leading edge of the population to see if NeabNPV application at this front can delay or halt the advance of the BFS population.

Attachments

Maps of proposed NeabNPV application sites.

Copy of Operators Licence (terms and conditions) from the Department of Environment and Labour applicable to forest insecticide use.

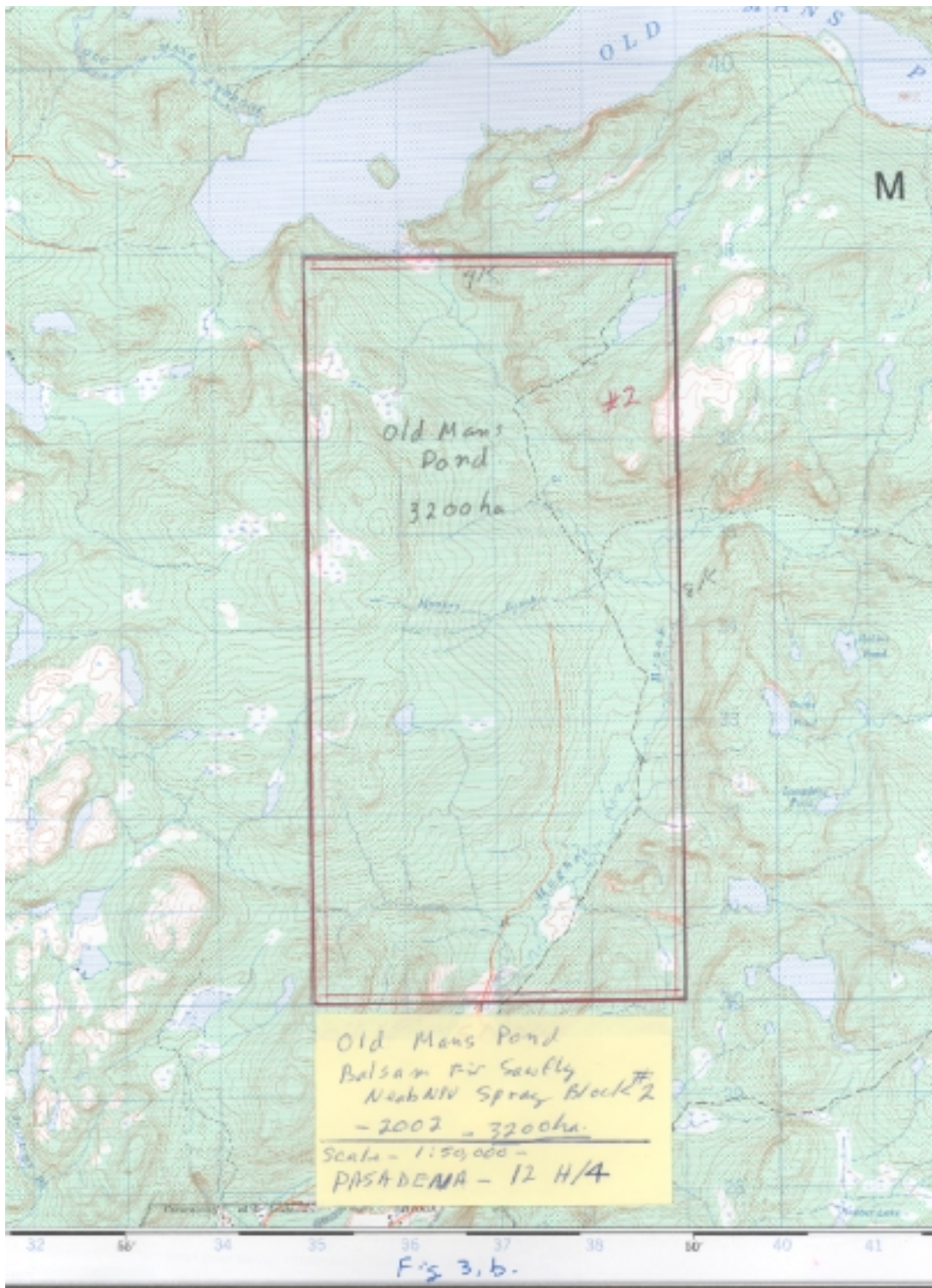
PMRA Research Permit.

Product label.



Figure 3a. Proposed area of 2002 aerial application of NeabNPV, block 201(red), in western Newfoundland. These are block boundaries. Buffer (no spray) zones inside the proposed area will be used, as per the Operator's Licence from the Department of Environment.





Roads	Routes	Oil highway	Highway 2 lane
hard surface	revêtement dur	single lane	route à 1 voie
hard surface	revêtement dur	2 lanes	route à 2 voies
soils or stabilized surface, all weather	gravier, aggrégat de, boue stabilisé	3 lanes	route à 3 voies
SOIL SURFACE, dry weather	de gravier, tamponnet	4 lanes or more	route à 4 voies
unimproved road or street	route non stabilisé au sol	5 lanes or more	route à 5 voies
GRAVEL	de sable		
RAILROAD	voies ferrées		
RAILROAD	voies ferrées		

FOR COMPLETE REFERENCE SEE REVERSE SIDE POUR UNE LISTE COMPLÈTE DES SIGNES, VOIR LE VERSO

Label **EXPERIMENTAL USE ONLY**

ABIETIVIRUS

Flowable Biological Insecticide

RESTRICTED For Use In Forestry

READ THE LABEL BEFORE USING

KEEP OUT OF REACH OF CHILDREN

GUARANTEE: *Neodiprion abietis Nucleopolyhedrovirus*, NeabNPV:

4 x 10⁹ viral occlusion bodies (OBs) per millilitre.

RESEARCH PERMIT NO: 38-RP-02 PEST CONTROL PRODUCTS ACT

Net Contents: 30 mL (1.2 x 10¹¹ OBs)

Not for sale.

Canadian Forest Service—Atlantic Forestry Centre

P. O. Box 4000

Fredericton, New Brunswick, E3B 5P7

Not for distribution to any person

Other than a researcher or

cooperator.

NOTICE TO USER: This control product is to be used only in accordance with the directions on this label. It is an offence under the *Pest Control Products Act* to use a control product under unsafe conditions.

NATURE OF RESTRICTION: This product is to be used only in the manner authorized. Consult local pesticide regulatory authorities about use permits which may be required.

RESTRICTED USE: For use against balsam fir sawfly larvae in forests.

DIRECTIONS: Treat when balsam fir sawfly larvae are feeding. Add contents to 300 L 20% aqueous solution of molasses. Spray balsam fir foliage at a rate of 2.5 L of mix/ha for 1 x 10⁹ OBs/ha. Provide a uniform deposit on foliage. Larvae must eat deposit of ABIETIVIRUS to be affected. Recommended droplet size is 100 µm. All applications will occur when larvae are at the first-instar stage of development.

PRECAUTIONS: KEEP OUT OF THE REACH OF CHILDREN. Potential sensitizer. Avoid contact with skin, eyes, or clothing. Wear a long-sleeved shirt, long pants, water-proof gloves, and eye goggles when handling, mixing/loading, or applying the product during all clean-up/repair activities. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

FIRST AID: In case of contact, flush skin or eyes with clean water. If irritation persists, obtain medical attention or contact a poison control centre.

DISPOSAL: Do not reuse container. Follow provincial instructions for any required cleaning of the container prior to its disposal. Make empty container unsuitable for use and dispose in accordance with provincial requirements. For information on the disposal of unused, unwanted product and the cleanup of spills, contact the provincial regulatory agency or the manufacturer.

Part 1.2 Product Profile and Proposed Use Patterns (from *Registration Guidelines for Microbial Pest Control Agents and Products*, Pro98–01).

i) *Neodiprion abietis* Nucleopolyhedrovirus NeabNPV Baculoviridae

ii) NeabNPV is a baculovirus within the genus *Nucleopolyhedrovirus* (NPV). NPVs are a large group of viruses with covalently closed, double-stranded DNA genomes of 88–153 kb (NeabNPV genome is approximately 95 kb). In NeabNPV, numerous virions are singly occluded within an occlusion body (OB) of polyhedrin. Polyhedrin is a 29-kd protein. Baculoviruses are restricted to arthropods, mostly insects. NPVs have a high degree of host specificity affecting a single insect species or ones that are closely related. Sawfly NPVs are especially host specific, and those described to date only seem to infect and replicate in the midgut epithelial cells of a single host species (Wallace and Cunningham 1995). Sawfly NPVs (including NeabNPV) are ingested by host larvae. Polyhedrin is dissolved in the gut releasing the virions. The virions fuse with the microvilli of the midgut epithelial cells and nucleocapsids are transported into the nucleus where they uncoat and undergo replication. Viral morphogenesis occurs in the nucleus and, eventually, the host cell dies and lyses releasing OBs into the gut lumen of the host. OBs pass out with the frass and are consumed by other host larvae. NeabNPV was isolated from balsam fir sawfly (*Neodiprion abietis*) larvae collected near Corner Brook, Newfoundland.

iii) The recommended application rate of sawfly NPVs is $1-5 \times 10^9$ OBs/ha (see also Wallace and Cunningham 1995).

iv) Bioinsecticide (larvicide).

v) Domestic.

vi) Formulated in 20% aqueous molasses.

vii) Control of balsam fir sawfly (*Neodiprion abietis*)

viii) Site of application–balsam fir forest stands in western Newfoundland near Corner Brook.

ix) Not applicable

x) 1×10^9 OBs/ha mixed with 20% aqueous molasses in a volume of 2.5 L/ha.

xi) Single application to coincide with first- and second larval instars, mid-June to early July.

xii) Fix-winged aircraft equipped with Micronaire AU 4000 nozzles.

xiii) Standard procedures for aerial applications (protective clothing, eyewear, etc.) to be followed.

xiv) Sawfly NPVs are not known to infect or affect any organisms other than their specific host.

5.0 Data Requirements

5.1 Agent Specifications and Characteristics (*Part 2 of Registration Guidelines for Microbial Pest Control Agents and Products*, Pro98–01).

Part 2.0 Product Characterization and Analysis

Part 2.1 Natural Resources Canada

Canadian Forest Service–Atlantic Forestry Centre
1350 Regent Street
P.O. Box 4000
Fredericton, New Brunswick, E3B 5P7

Part 2.2 Natural Resources Canada

Canadian Forest Service–Atlantic Forestry Centre
1350 Regent Street
P.O. Box 4000
Fredericton, New Brunswick, E3B 5P7

Part 2.3 Natural Resources Canada

Canadian Forest Service–Atlantic Forestry Centre
1350 Regent Street
P.O. Box 4000
Fredericton, New Brunswick, E3B 5P7

Part 2.4 ABIETIVIRUS

Part 2.5 *Neodiprion abietis Nucleopolyhedrovirus* NeabNPV Baculoviridae

Part 2.6 None.

Part 2.7 Characterization of the MPCA

Part 2.7.1 Origin, Derivation and Identification of MPCA

i) *Neodiprion abietis Nucleopolyhedrovirus* NeabNPV Baculoviridae
(Volkman et al. 1995).

ii) Balsam fir sawfly nuclear polyhedrosis virus (Olofsson 1972).

iii) NeabNPV.

iv) In August 1997, balsam fir sawfly larvae were collected from two plots near Corner Brook, Newfoundland. These insects were reared in our laboratory in Fredericton, New Brunswick, and larvae that died in rearing were examined for the presence of NeabNPV. This virus was found in a number of larvae and was isolated. Virus amplification of NeabNPV was

carried out at the Pasadena Field Station in July and August of 1998 and 1999. Here, larvae were reared on balsam fir foliage in 5-L plastic tubs. Virus was applied to the foliage and dead insects were picked from the foliage by hand and frozen.

v) Stock isolates of NeabNPV are held at either 4°C or –20°C.

vi) Not applicable.

Part 2.7.2 Biological Properties of the MPCA

i) NeabNPV has been reported in populations of BFS from Alberta, Saskatchewan, Manitoba, Ontario, Quebec, Ontario (see Olofsson 1972) and Newfoundland (here). BFS feed on one-year-old and older foliage of balsam fir (*Abietis balsamea*).

ii) Balsam fir sawfly, *Neodiprion abietis* (Hymenoptera: Diprionidae). Larval infection is *per os*. NeabNPV virions infect the cells of the larval gut. Viral replication only occurs in the nucleus of midgut epithelial cells (Federici 1993).

iii) NeabNPV is known to infect and replicate only in the midgut cells of BFS larvae.

iv) Sawfly NPVs infect only 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 they can infect other insects. Host death normally occurs within a week but, during that time, the host is producing infective units of the disease.

v) No plasmids or extra chromosomal DNA. NeabNPV is a naturally occurring pathogen of the BFS and was isolated from that host. This virus has not been subjected to any type of nucleic acid recombination.

vi) NeabNPV can only be produced *in vivo*, in BFS larvae. There are no tissue culture systems available for the production of NeabNPV. Like most baculoviruses, NeabNPV is probably sensitive to UV radiation.

vii) No unusual characteristics, morphological, physiological, biochemical or otherwise.

viii) Experimental ground applications of NeabNPV were made against BFS in Ontario in the early 1970s (Olofsson 1972). Other sawfly viruses have been used, successfully in trials against sawfly pests (Wallace and Cunningham 1995). Two are currently registered in Canada for use to control sawfly pests: Sertifivirus (*Neodiprion sertifer Nucleopolyhedrovirus*, NeseNPV) for European spruce sawfly (*Neodiprion sertifer*) and Lecontivirus (*Neodiprion lecontei Nucleopolyhedrovirus*, NeleNPV) for redheaded pine sawfly (*Neodiprion lecontei*) (see Wallace and Cunningham 1995).

5.2 Human Health Safety Testing

2) Medium scale field trial up to 5000 ha forestry

Baculoviruses are known to infect only arthropods, mostly insects. They are not related to any known vertebrate or plant pathogenic viruses. Specificity and safety tests of baculoviruses have shown that there are no toxicological or other deleterious effects to mammals, birds, aquatic organisms, beneficial or other nontarget insects.

Literature on specificity and safety testing of baculoviruses up to 1985 is reviewed by Gröner (1986) and to 1989 by Laird et al. (1990). Additional literature from 1990 to the present was sought using searches of Current Contents, Agricola and the Canadian Research Index. No reports were found attributing any adverse effects of baculoviruses on nontarget organisms.

Part 2.8 Manufacturing Methods and Quality Assurance (*Registration Guidelines for Microbial Pest Control Agents and Products, Pro98-01*).

a) Preservation and Maintenance of the Productive Strain

BFS larvae infected with NeabNPV from the original collection site have been frozen and stored at -20°C . OBs isolated from host larvae are stored at 4°C in 10% NaCl (w/v) to retard bacterial growth. To prevent cross-contamination with NeabNPV from other locations, NeabNPV-NF is produced only in BFS collected from the region around the site of the original isolation in western Newfoundland.

b) Manufacturing Processes

Virus amplification of NeabNPV was carried out at the Pasadena Field Station in July and August 1998. Here, larvae were reared on balsam fir foliage in 5-L plastic tubs. Virus was applied to the foliage and dead insects were picked from the foliage by hand and frozen. For additional virus amplification, balsam fir foliage with BFS eggs was collected near Corner Brook between March and June 1999 and shipped to Fredericton. Larvae were hatched in the laboratory and were infected with NeabNPV. Purification of NeabNPV produced in summer 1998 and winter and spring 1999 was completed by the end of June 1999 (see method below).

In July 1999, NeabNPV was mixed with 25% aqueous commercial grade molasses and 3% (v/v) sticker (XA Oil Concentrate, United Agri Product, Dorchester, Ontario, Pest Control Products Act registration number 11769). It was applied to balsam fir trees infested with BFS larvae at a location southeast of Big Gull Pond. Collections of larvae began at the first sign of larval mortality and continued for the next 10 days. Branch tips with infected larvae were cut from balsam fir and white spruce trees. These were placed into 50-lb brown paper bags so that the bags were half-filled with foliage. The bags were stapled shut and the larvae were left to die or finish their development in these bags in the laboratory at the Pasadena Field Station at ambient temperatures. Approximately 600 bags of foliage were collected. Dead larvae from the bags were removed from the branches by hand, placed in 50-mL centrifuge tubes and frozen. In all, 60 centrifuge tubes were filled with dead, infected larvae. NeabNPV from this material was purified using the method described below. This virus was applied in the field trials carried out in 2000 and 2001. BFS larvae from the 2001 spray blocks were collected, reared and processed as described for the 1999 production.

NeabNPV isolation protocol — large scale

1. Thaw and re-hydrate NPV infected insects in water.
2. Homogenize insects in a 1000 mL beaker using a hand held blender.
3. Dilute with water and add 1% SDS to a concentration of 0.3% (final volume approximately 10 times the insect volume).
4. Add magnetic stirrer bar and stir for 60 min.
5. Filter through plastic mesh, save filtrate (contains the virus).
6. Resuspend solid debris in 0.3% SDS and stir 5 min.
7. Filter again through plastic mesh and repeat until clear filtrate is obtained.
8. Filter virus solution through 8 layers of cheesecloth.
9. Centrifuge for 15 min in a Sorvall RC 28S centrifuge and HS-4 rotor (approx. 2000xg).
10. Discard supernatant and add more of the virus solution to centrifuge tubes; repeat steps 9 and 10 until all virus solution used.
11. Resuspend virus pellets in 0.3% SDS and vortex.
12. Repeat centrifugation and resuspension until a clear supernatant is obtained.
13. Pool pellets.
14. Resuspend pellet in 0.5 M NaCl, centrifuge.
15. Resuspend the pellet in a small volume of water.

NeabNPV suspensions are centrifuged and stored in 10% NaCl at 4°C to reduce the amounts of contaminating bacteria. Dilution plates of the suspension will be made on selective nutrient agars to isolate bacteria (Research and Productivity Council) (analysis attached). To further reduce unwanted bacterial propagation, viral suspensions are to be added to adjuvants in the field immediately prior to use.

OBs are quantified by combining a known volume of an unknown concentration of NeabNPV OBs with a known volume of a known concentration of latex beads. Latex beads and OBs from several fields of view are counted under the 100x oil lens of a compound microscope. The concentration of OBs is determined as a proportion to the number of latex beads counted.

5.3 Food and Feed Residue Studies

Not applicable.

5.4 Environmental Fate and Environmental Toxicology

IR I: NeabNPV was isolated from western Newfoundland in the same ecozone (Zone 5) where it will be used.

References

- Federici, B.A. 1993. Viral pathobiology in relation to insect control. *In*: N.E. Beckage, S.N. Thompson and B.A. Federici (editors). Parasites and pathogens of insects Vol. 2: Pathogens. Academic Press, San Diego, California. pp. 81–101.
- Gröner, A. 1986. Specificity and safety of baculoviruses. *In*: R.R. Granados and B.A. Federici (editors). The biology of baculoviruses Vol. II: Practical application for insect control. CRC Press, Boca Raton, Florida. pp.177–202.
- Laird, M., L.A. Lacey and E.W. Davidson. 1990. Safety of microbial insecticides. CRC Press, Boca Raton, Florida.
- Olofsson, E. 1973. Evaluation of a nuclear polyhedrosis virus as an agent for the control of the balsam fir sawfly, *Neodiprion abietis* Harr. Insect Pathology Research Institute, Department of the Environment, Canadian Forestry Service, Sault Ste. Marie, Ontario, Information Report IP-X-2.
- Piense, H., D.P. Ostaff and E.S. Eveleigh. 2001. Growth loss and recovery following defoliation by the balsam fir sawfly in young, spaced balsam fir stands. *Can. Ent.* 133: 675–686.
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- Wallace, D.R. and J.C. Cunningham. 1995. Diprionid sawflies. *In*: J.A. Armstrong and W.G.H. Ives (editors). Forest Insect Pests in Canada. Natural Resources Canada, Ottawa, Ontario. pp. 193–232.



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Pest
Management
Regulatory
Agency

Agence de
réglementation
de la lutte
antiparasitaire



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Fax / Télécopieur: (613) 736-3770

E-mail / C. élec:

Lynda_Austen@hc-sc.gc.ca

FACSIMILE TRANSMITTAL NOTICE

AVIS DE TÉLÉCOPIE

TO / À: Ed Kettela, Atlantic Forestry Centre
FAX / TÉLÉC.: 506-452-3214

FROM / DE: Lynda Austen
Efficacy and Sustainability Assessment Division

DATE: May 17, 2002

SUBJECT / OBJET: Abietivirus research permit NFLD TRIALS

No. of pages including this page / Nombre de pages incluant celle-ci : 5

Mr Kettela;

Chris Lucarotti asked me to FAX information to your attention once this research permit was granted.

Hard copy (addressed to Chris Lucarotti) will follow by mail.

Canada

05/17/2002 11:03 FAX 613 736 3770

PMRA

003

Health
CanadaSanté
CanadaPest
Management
Regulatory
AgencyAgence de
réglementation
de la lutte
antiparasitaire

A.L. 6607E
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TEL: (613) 736-3780
FAX: (613) 736-3770
e-mail: Richard_Aucoin@hc-sc.gc.ca

834.1 RP
Sub. No. 2002-0228

May 17, 2002

by fax: (506) 452-3525 (original by mail)

Christopher Lucarotti, Ph.D.
Research Scientist
Canadian Forestry Service, Atlantic Forestry Centre
P.O. Box 4000
Fredericton, NB E3B 5P7

Dear Dr. Lucarotti:

**Re: Sub. No. 2002-0228 Research Permit Application for Abietivirus Biological Insecticide,
Containing Active Ingredient balsam fir sawfly nucleopolyhedrovirus (NUV)**

The Pest Management Regulatory Agency (PMRA) has now completed the review of your application for a Research Permit of January 16, 2002 which was received on January 24, 2002. Your application is acceptable and Research Permit Number 38-RP-02 has been assigned to Research Permit Submission Number 2002-0228.

The following conditions and comments apply to this research permit:

Label Revisions:

- Under **"DIRECTIONS"** add the names of the pests and tree hosts. In addition, state that applications are to occur when larvae are at the first instar stage of development.
- The following text is required under **"PRECAUTIONS"**:
 - **Potential sensitizer. Avoid contact with skin, eyes, or clothing. Wear a long-sleeved shirt, long pants, water-proof gloves, and eye goggles when handling, mixing/loading or applying the product and during all clean-up/repair activities. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.**

Page 1 of 2

Canada

Other Requirements:

- Maps indicating test sites must be submitted prior to the first application.
- Signs must be posted to restrict entry to all test sites.
- Please revise the Product Specification Form (PSF) to confirm that the purified OBs are suspended in a 10% NaCl solution as opposed to water (as indicated on the PSF submitted).

Comments on Quality of Submission:

- For future submissions, please include all relevant information regarding microbial contaminant analysis testing (e.g., identify samples, describe methods).

A copy of your experimental label is enclosed along with the Research Permit. Posters listing warnings, permit number and a contact person must be posted at each research site (refer to the attached pages for further information on this).

Should you have any questions or comments regarding this review please do not hesitate to contact the Administrative Coordinator for this submission, Lynda Austen, at 613 736-3784.

Yours sincerely,



Richard Aucoin, Ph.D.
Director
Efficacy and Sustainability Assessment Division

Attachments

cc Barbara Szegvary, CROS, Compliance, Lab Services and Regional Operations Division
(CLSROD)
Neil McTiernan, Regional Manager, Atlantic Region, CLSROD *for use in Newfoundland*

WARE SEARCH PERMIT2002-0228 review letter to applicant.wpd

F

Label

EXPERIMENTAL USE ONLY

ABIETIVIRUS

Flowable Biological Insecticide

RESTRICTED For Use In Forestry

**READ THE LABEL BEFORE USING
KEEP OUT OF REACH OF CHILDREN**

GUARANTEE: *Neodiprion abietis* Nucleopolyhedrovirus, NeabNPV:
1 x 10⁹ viral occlusion bodies (OBs) per millilitre.

RESEARCH PERMIT 38-RP-02

REGISTRATION NO: ~~XXXXXXXXXX~~ **PEST CONTROL PRODUCTS ACT**

Net Contents: 30 mL (1.2 x 10¹¹ OBs)

Not for sale.
Not for distribution to any person
other than a researcher or cooperator

Canadian Forest Service – Atlantic Forestry Centre
P. O. Box 4000
Fredericton, New Brunswick, E3B 5P7


NOTICE TO USER: This control product is to be used only in accordance with the directions on this label. It is an offence under the *Pest Control Products Act* to use a control product act under unsafe conditions.
NATURE OF RESTRICTION: This product is to be used only in the manner authorized. Consult local pesticide regulatory authorities about use permits which may be required.
RESTRICTED USE: For use against balsam fir sawfly larvae in forests.
DIRECTIONS: Treat when larvae are feeding. Add contents to 300 L 20% aqueous solution of insecticides. Spray foliage at a rate of 2.5L of mix/ha for 1 x 10⁹ OBs/ha. Provide a uniform deposit on foliage. Larvae must eat deposit of ABIETIVIRUS to be affected. Recommended droplet size is 100 µm.

PRECAUTIONS: **KEEP OUT OF THE REACH OF CHILDREN.** Avoid contact with skin, eyes and clothing. Wash with soap and water after use.

FIRST AID: In case of contact, flush skin or eyes with clean water. If irritation persists, obtain medical attention or contact a poison control centre.

DISPOSAL: Do not reuse container. Follow provincial instructions for any required cleaning of the container prior to its disposal. Make empty container unsuitable for use and dispose in accordance with provincial requirements. For information on the disposal of unused, unwanted product and the cleanup of spills, contact the provincial regulatory agency or the manufacturer.

Consult accompanying letter for other additions
and revisions to the label.



APPLICATION FOR RESEARCH PERMIT
DEMANDE DE PERMIS DE RECHERCHE

Under the Pest Control Products Act / Aux termes de la Loi sur les Produits antiparasitaires
 NOTE: TYPE OR PRINT CLEARLY, LEAVE SHADED AREAS BLANK. / NOTA: ECRIRE LISIBLEMENT OU DACTYLOGRAPHIER. NE PAS ECRIRE DANS LES ESPACES OMBRES.

1. Brand name or experimental no./Marque de commerce ou n° d'essai du produit Abietivirus	2. Reg. no. if appl./N° d'enreg. si appl.
3. CSA common name-Chemical name and percent for each active ingredient/Nom commun (ACNOR) formule chimique et pourcentage de chaque matière active Balsam fir sawfly nucleopolyhedrovirus (NeabNPV)	
4. Name of applicant/Nom du demandeur Dr. Christopher Lucarotti	
Address/Adresse Natural Resources Canada Canadian Forest Service-Atlantic Forestry Centre 1350 Regent Street P.O. Box 400 Fredericton NB E3B 5P7	
5. Name of research coordinator/Nom du co-ordinateur de la recherche Dr. Christopher Lucarotti	
Address/Adresse as above	
6. Name of supplier/Nom du fournisseur Dr. Christopher Lucarotti	
Address/Adresse as above	

7. Quantity of product distributed or obtained by region/Quantité de produit distribué ou obtenu par région N/A	7B. Points of entry/Points d'entrée N/A
8. Host or crop being treated/Hôte ou culture étant traitée balsam fir Abies balsamea	8F. Additional directions/Directives supplémentaires Add 10ml of NeabNPV-Abietivirus suspension to each 100h of 20% aqueous molasses
9. Pest/Agent nuisible Balsam fir sawfly Neodiprion abietis Hymenoptera Diprionidae.	9. Preharvest interval/Intervalle de sécurité N/A
10. Rate/Taux 1x10⁹ occlusion bodies /ha 2.5h of mix /ha	10. Purpose/But <input type="checkbox"/> Residue <input checked="" type="checkbox"/> Efficacy <input type="checkbox"/> Environment <input type="checkbox"/> Other
11. Timing and number of applications (testing dates where possible) / Intervalles et nombre d'applications (dates d'applications lorsque possible) One application each block June 15-30 2002	11. Exact location, size & number/Région exacte, grandeur & numéro Newfoundland near Corner Brook (block 20) and the Bay d'Espoir - other blocks besides 20 to be selected once egg counts completed Total up to 5000h
12. Application equipment to be used/Équipement d'application à être utilisé Cessna 188 AgTrucks with 4 Micron Air All 4000 atomizers	12. Type of research/Genre de recherche <input type="checkbox"/> New use of reg. product <input type="checkbox"/> New formulation <input checked="" type="checkbox"/> New active/New source

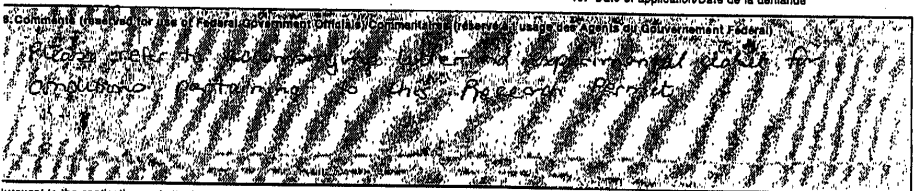
13. Indicate if the following have been submitted/indiquer si les renseignements suivants ont été soumis

A. Specification form / Formulaire de spécification	<input checked="" type="checkbox"/> Y/O <input type="checkbox"/> N/N	C. Safety data sheets / Bulletins de données sur l'innocuité	<input checked="" type="checkbox"/> Y/O <input type="checkbox"/> N/N	E. List of cooperators and researchers / Liste de co-opérateurs et de chercheurs	<input checked="" type="checkbox"/> Y/O <input type="checkbox"/> N/N	G. Data (specify) / Données (spécifier)	<input checked="" type="checkbox"/> Y/O <input type="checkbox"/> N/N
F. Experimental label / Étiquette expérimentale	<input checked="" type="checkbox"/> Y/O <input type="checkbox"/> N/N	D. Maps/Cartes	<input checked="" type="checkbox"/> Y/O <input type="checkbox"/> N/N	F. Protocols/Protocoles	<input checked="" type="checkbox"/> Y/O <input type="checkbox"/> N/N	H. Other (specify) / Autres (spécifier)	<input checked="" type="checkbox"/> Y/O <input type="checkbox"/> N/N

I, the undersigned, hereby specify that the above information is correct in all respects, I understand that the grant of a permit does not create any liability on the Crown and the applicant remains totally liable for such things as damage to treated crops or properties on the crops or property of others and for such matters as occupational health and safety and environmental impact as a result of this research being performed.

Lucarotti
 Signature of applicant/Signature du demandeur

January 15 2002
 15. Date of application/Date de la demande



In pursuance to the application and attachments and subject to amendments (if any) made therein, authorization is granted to proceed with research involving the specified pest control product. This issuance of this federal permit does not imply provincial approval. It is the applicant's responsibility to obtain provincial approval for pesticide research trials where this may be required. Crops harvested from treated plots should not be sold for food purposes unless written authorization is obtained from the Food Directorate of Health Canada.

This authorization of research for the year ending: / Cette présente autorisation prend fin le: 2002 31 DEC. 31	Reviewing Officer/Agent de révision: <i>[Signature]</i>	Code	Date: May 17/02
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