Experimental Application Of The Balsam Fir Sawfly Nucleopolyhedrovirus (Abietiv™) Against Its Natural Host, The Balsam Fir Sawfly

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Nature of Proposed Pesticide Application

The Province of Newfoundland and Labrador continues to face serious and widespread infestations of balsam fir sawfly (*Neodiprion abietis* – Hymenoptera: Diprionidae). These infestations are threatening substantial investments in silviculture and consequently the long-term wood supply for the forest industry. For a fifth year, the Canadian Forest Service (CFS), in co-operation with the Newfoundland and Labrador Department of Natural Resources (NLDNR) and Forest Protection Limited (FPL), 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 forecast to receive moderate to severe the balsam fir sawfly defoliation in 2005 and at the leading edge of the infestation. Applications of this biological control agent, which goes by the registered trade name, Abietiv, will be made using fixed-wing aircraft and/or helicopters.

Description of Balsam Fir Sawfly Problem

Insect population levels

The balsam fir sawfly is native to and has been an occasional pest on balsam fir in Newfoundland. Recently, it has become more important as a pest of young and semi-mature balsam fir (*Abies balsamea*), 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 eggs are laid in the needles of the current year. Populations of balsam fir sawflies are normally regulated by natural pathogens, parasites and predators. Population outbreaks have normally been of short duration (3 - 4 years) and were terminated by natural factors, predominantly NeabNPV. Although localized damage was often severe, tree mortality was 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 infestation in western Newfoundland was detected in 1991 near Bottom Brook, east of Stephenville. By 1994, approximately 1,216 hectares (ha) of defoliation were recorded. In 1995, high population levels were observed. Moderate and severe defoliation was mapped on 12,600 ha, with some 10 percent mortality occurring in young fir stands. The situation in 1996 saw the infestation continue to expand 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 balsam fir (PCT) stands. A total of 53,000 ha were defoliated in 1997 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 totaled approximately 24,400 ha with 16,500 ha occurring in western Newfoundland, 5,800 ha in Bay d'Espoir and 2,100 ha on the Burin Peninsula. In 1999, moderate and severe defoliation occurred on 18,400 ha with 12,400 ha in western Newfoundland, 3,300 ha in Bay d'Espoir and 2,800 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 9,000 ha in the Bay d'Espoir in 2001.

In 2002-2004, moderate to severe defoliation reached 60,000 ha in western and southern Newfoundland. The western area extended from south of Grand Lake, north to Old Man's Pond and from Stag Lake-Cook's Brook across the Humber Arm near Gillams and east to Steady Brook-Corner Brook Lake. This area is of particular concern because a significant proportion is PCT. These PCTs have been established, at an average cost of $1,000+/ha (a total amount in excess of $10 million). These 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 south-west 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 balsam fir sawfly populations. With prolonged, severe defoliation, affected trees will be stressed, lose growth and be subject to mortality from secondary insects and diseases. It is estimated that, since the balsam fir sawfly outbreak began, the Province has lost is excess of 2 m^3 of growth per hectare per year, a loss in excess of 120,000 m^3 of incremental growth.

Control Options

A pest management program is being developed against the balsam fir sawfly in Newfoundland to protect valuable young stands and silviculturally treated areas of balsam fir. The purpose of the program is to reduce balsam fir sawfly population levels in treated areas to minimize the loss of foliage, tree growth and to prevent tree mortality due to secondary infestations in trees weakened by balsam fir sawfly attack. Unfortunately, control options for balsam fir sawfly are limited. Experimental programs have been carried out by CFS and its collaborators in 1998, 1999, 2000, 2001, 2002, 2003 and 2004 in Newfoundland and in other jurisdictions to develop biological control options for a number of sawflies, including the balsam fir sawfly, yellowheaded spruce sawfly (*Pikonema alaskensis*), pine false webworm (*Acantholyda erythrocephala*) and introduced pine sawfly (*Diprion similis*). Progress has been made and work is continuing.

Dylox

The organophosphate insecticide, Dylox 420 (trichlorfon) is no longer considered as an option. It was used in 1998 under an emergency registration from the Pest Management Regulatory Agency (PMRA) of Health Canada. Based on experimental trials conducted in the same year, it was determined that lower dosages than those recommended could be effective. NLDNR requested registration of Dylox and, for 1999 only, PMRA granted a temporary registration for Dylox for use against balsam fir sawfly. There were a number of conditions related to buffer zones (no spray zones), dose parameters and monitoring requirements attached to the temporary registration.

Dylox is fully registered for use against the yellowheaded spruce sawfly but is not being pursued further for balsam fir sawfly. This is due to 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 provincially. This restricted control measures to approximately 3,100 ha. 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 v*ar. *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 insect host. 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. Sawflies belong to the order Hymentoptera (includes 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 larval mosquitoes and blackflies not those of lepidopteran larvae. In 1999, B.t.i. was tested experimentally against balsam fir sawfly on a small area. B.t.i. was found not to be effective.

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 balsam fir sawfly. 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 may be applied aerially and/or from the ground to horticultural-ornamental plants, trees, shrubs and agricultural crops. An operational program using Neemix 4.5 was carried out by NLDNR on about 1,500 ha in the Bay d'Espoir in 2001. In 2002, Neemix 4.5 was applied to just over 6,000 ha near Corner Brook. Neemix 4.5 was not available for use in 2003 or 2004 because the temporary registration has expired and it will not be available in 2005.

Balsam fir sawfly nucleopolyhedrovirus

Nucleopolyhedroviruses (NPVs) are a large group of viruses with covalently closed, double-stranded DNA genomes of 88-153 kilobases. 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 on mammals, birds, amphibians, aquatic

microorganisms and beneficial and other non-target insects. Population crashes due to NPV epidemics occur in many species of sawflies. NPVs are transmitted through ingestion by a suitable host larva. Viral polyhedral inclusion bodies (PIBs) 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 PIBs are sloughed off into the frass and out of the body where they can infect other host insects. Death normally occurs within 1 to 2 weeks but, during that time, 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.

Progress 1997-2004

Field trials

In 1999, NeabNPV (Abietiv) was applied to 1 ha of balsam fir forest in order to field amplify the virus. From this 1-ha application, enough NeabNPV was obtained to treat 1,800 ha of forest at an application rate of 1 x 10^9 PIBs/ha. On July 22-23, 2000, three blocks, each 50 ha in area, between Pinchgut Lake and Big Gull Pond near Corner Brook, Newfoundland, were treated aerially with Abietiv at 3 x 10^9 PIBs/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 Micronair AU4000 atomizers). Aerial field trails (1 x 10^9 PIBs/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 PIBs/ha) in three blocks to the south, east and north of Corner Brook. [The results of efficacy trials carried out between 2000 and 2002 were recently published (Moreau et al. 2005) and a copy is attached to this document.] In 2003 and again in 2004, Abietiv was applied to areas totaling approximately 5,000 ha in each year. The locations of these application plots were around Old Man's Pond, north of Deer Lake across from Pasadena and to the south west of Pasadena. In all trials in all years, there was good deposit on the targeted areas resulting in higher levels of NeabNPV infection in larval populations in the spray blocks compared to the control blocks. Additionally, it was generally found that the number of balsam fir sawfly pupae and eggs was lower in the spray blocks compared to the control blocks in the year of the spray. In the year immediately following Abietiv applications, it was 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. An objective of the 2002 field trial was to determine if application of Abietiv against first-instar balsam fir sawfly would kill the insects in time to give some foliage protection in the year of application. There was some foliage protection but it was limited.

In the Abietiv field trials to date, we have found that i) Abietiv 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 PIBs/ha against first- and second-instar larvae results in increased levels of NeabNPV infection in larval populations within 15 days and iv)

application in one year can affect the population of balsam fir sawfly larvae in the next year resulting in significantly decreased defoliation.

Proposed Field Trials for 2005

The balsam fir sawfly infestation was apparent in Corner Brook and on the north side of the Humber Arm (Summerside – Hughes Brook) in 2001. In 2002, the infestation had spread further towards the northeast and the balsam fir sawfly infestation near Old Man's Pond was sprayed with Abietiv. On the other side of the Humber River, defoliation could be seen up the valley past Steady Brook towards Little Rapids. In 2003 and 2004 the balsam fir sawfly infestation has continued to spread eastward towards the Town of Deer Lake on either side of Deer Lake itself.

In 2005, Abietiv will be applied against first- and second-instar balsam fir sawfly larvae at a rate of 1×10^9 PIBs/ha. A 20% aqueous solution of molasses will be used as the carrier for the virus and the mixture will be applied at a rate of 2.5 L/ha using fixed-wing spray aircraft (Cessna 188 AgTrucks or Air Tractor AT-802s) equipped with Micronair AU4000 atomizers. Spray areas (Fig. 1) will be monitored for balsam fir sawfly eggs in fall 2005 and 2006 to determine the impact of Abietiv on balsam fir sawfly populations.

Separate spray blocks will be established for the production of Abietiv in areas where there is high balsam fir sawfly larval densities. These blocks will be sprayed when the larval index is peak second instar. Infected larvae will be collected and NeabNPV purified using established methods.

CFS and its cooperators utilize appropriate current equipment and technology. CFS complies with existing regulatory guidelines. Parameters of the spray blocks will be determined on the ground using hand-held GPS personal navigators. Block coordinates will be transferred computers on board the aircraft. These aircraft are equipped with the latest navigational and spray equipment including mapping (GIS) and positioning (GPS) systems, automatic on/off spray-boom controls, variable speed-pressure-flow monitors and controllers, real-time, on-board meteorological sensors and radar altimeters. At the time of the spray application, this equipment and related application software ensure the highest level of accuracy of application currently available. CFS personnel will assesses the favorability of weather parameters before and during spray application. To ensure environmental safety, spray bases will have available 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 Abietiv formulation (mixer/loader) will wear the required safety equipment as indicated on the experimental label during mixing and loading onto the aircraft. In addition, approved safety precautions and established rules and guidelines will be adhered to concerning personal hygiene of all mixer/loader personnel working with Abietiv 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 2004, spraying near habitation will be subject to terms and conditions of the Operator's Licence from the Department of Environment & Conservation 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 is 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 & Conservation will be followed. These include the reporting of any incidents, such as spills, to the appropriate authorities. In connection with this, CFS and NLDNR have contingency plans that are reviewed and approved annually prior to receiving of an Operator's Licence. These plans outline procedures for spill reporting, emergency first aid for exposure, insecticide spill only, 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/or news releases and through appropriate direct contact if required. Information included will be the product being used, general areas of spray blocks, timing of application, contact numbers, etc. Access roads to the general 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 and the general public can call to find out the status of areas receiving treatment.

Regional offices of the NLDNR and the Department of Environment and Conservation, as applicable, will be provided with maps showing spray blocks. These maps will be made available for viewing by the general public during regular office hours. District offices of the NLDNR will be made aware of spray blocks in their area and are 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 get Abietiv registered for operational use against the balsam fir sawfly, will be present in Newfoundland during the spray period. 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; for example, proximity to habitation, water supply areas, recreational areas (fishing, camping, berry picking) 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 which 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, NLDNR, Corner Brook Pulp and Paper Ltd. and Abitibi-Consolidated Inc. was initiated to investigate the ecology of the balsam fir sawfly. The prevalence of natural control factors such as viruses, fungi and parasites and their effect on balsam fir sawfly populations are being investigated. The impact of the balsam fir sawfly on and differences observed between thinned and unthinned stands is also being investigated. In 1998, additional financial resources were obtained through a Natural Sciences and Engineering Research Council (NSERC) – CFS – Industry grant which is administered through the University of New Brunswick. This funding continued through 2001. Funding for 2000-2005 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 and CFS Enhanced Pest Management for the period 2001-2006. These cooperative research programs, in identifying natural factors that influence balsam fir sawfly populations, will hopefully lead to an integrated pest management strategy against this pest.

In November 2000, CFS research staff had a registration pre-submission consultation with officials from PMRA. The purpose of the consultation was to determine the requirements that would have to be met in order to get a registration for the operational use of Abietiv. A great deal of progress has been made since then including: i) five years of field efficacy trials, ii) six years of field work by three graduate students studying balsam fir sawfly ecology, iii) the NeabNPV genome has been fully sequenced, iv) bioassays against non-target insects and *Daphnia magna* (freshwater invertebrate crustacean) and v) mammalian toxicology – pathogenicity tests have been carried out. NeabNPV did not have have any detectable effect on any of the insect species (other than sawflies), *Daphnia* or on the mammalian animals and cell lines tested. Documentation for the registration of Abietiv was submitted to PMRA in June 2004. In January 2005, PMRA officials asked Dr. Lucarotti for additional information about Abietiv and this additional information was sent to PMRA in April 2005.

Registration Approval Process

Any pest control product manufacturer who wishes to sell a pesticide in Canada must first register that product 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. A scientific evaluation of the product is then performed by PMRA. The scientific evaluation may take years, as the evaluation may require 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 reevaluation, 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, 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 Department of Environment and Conservation - Pesticides Control Section) are granted an applicator license and authorized to handle pesticides. The Pesticides Control Section is also responsible for compliance and enforcement activities.

The 2005 Abietiv research program will be regulated by the Pesticides Control Section of the Newfoundland and Labrador Department of Environment and Conservation.

Attachments

(1) Product Label (draft) with PMRA 2005 research permit number.
(2) Product Material Safety Data Sheet (draft).
(3) Moreau, G., Lucarotti, C. J., Kettela, E. G., Thurston, G. S., Holmes, S., Weaver, C., Levin, D. B. and Morin, B. 2005. Aerial application of nucleopolyhedrovirus induces decline in increasing and peaking populations of *Neodiprion abietis*. Biological Control 33: 65-73.