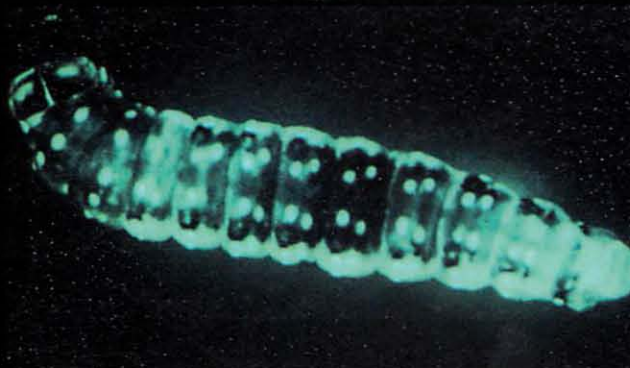
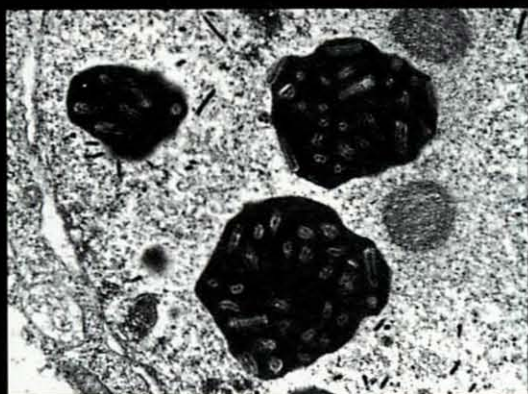


# *Pest Management Biotechnology*



## *Lutte biotechnologique contre les ravageurs*



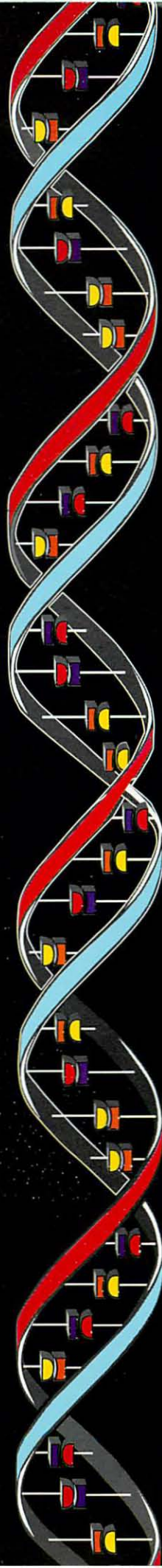
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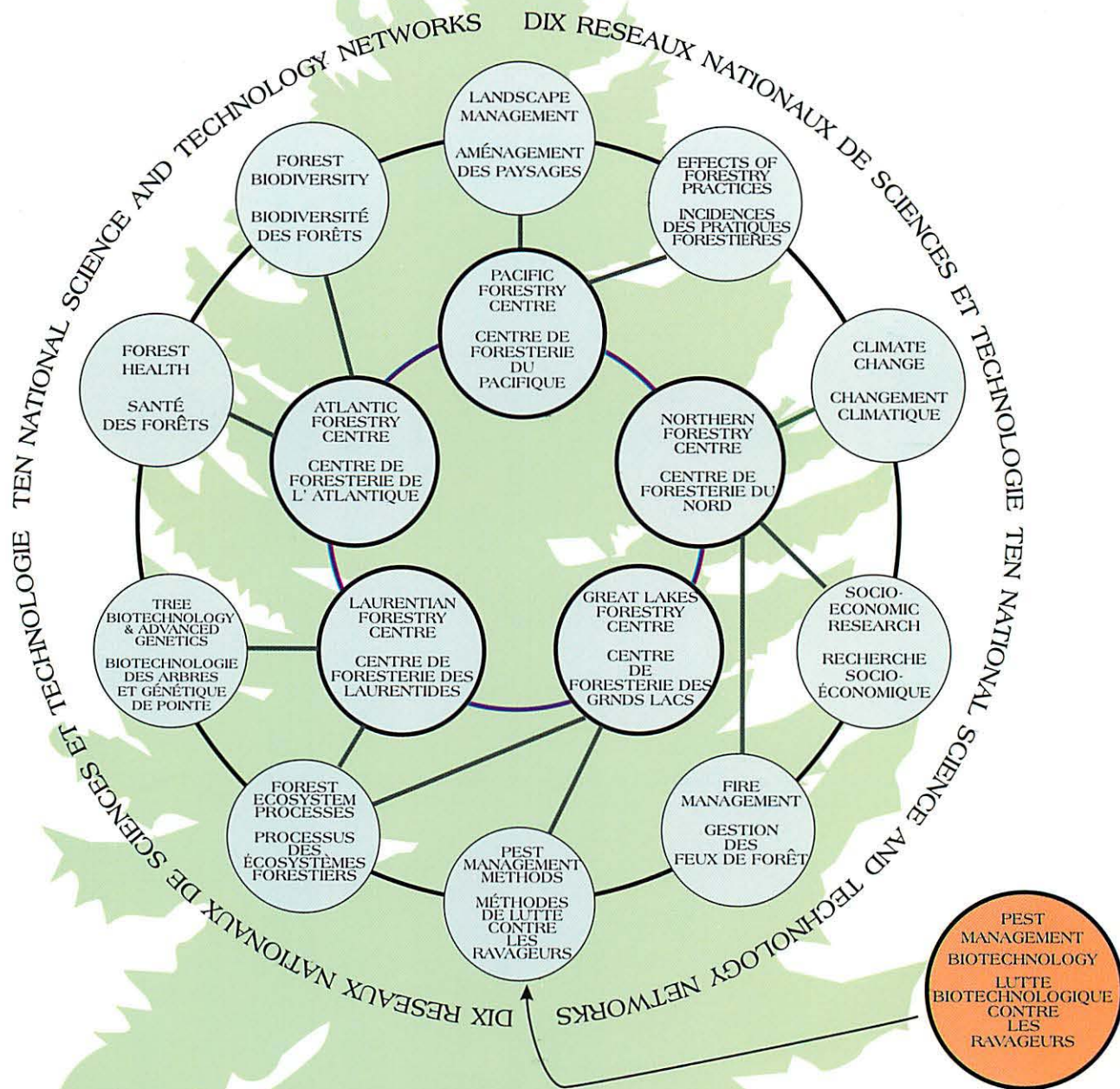
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# Canadian Forest Service Service Canadien des forêts



**For information on Pest Management Biotechnology contact / Pour information sur la lutte biotechnologique contre les ravageurs contactez :**

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# **Pest Management Biotechnology**

**Editor: Arthur Retnakaran**

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## **PREFACE**

Research in the Canadian Forest Service (CFS) has undergone profound changes in the last few years. The concept of networking and the team approach has been widely accepted as the way of the future. In 1993 Dr. Bill Cheliak, who was then the Program Manager at the Forest Pest Management Institute (FPMI), instituted such a team approach and formed the Pest Management Biotechnology Project consisting of Molecular Virology, Molecular Entomology, Insect Physiology, Tissue Culture and Virus Ecology. Since then FPMI has been amalgamated with the Great Lakes Forestry Centre and the Biotechnology Project has become a part of the national Pest Management Methods Network. The project has expanded to become national in scope, retaining its nerve center at the Great Lakes Forestry Centre in Sault Ste. Marie, Ontario. We are a fledgling project in a dynamic state, expanding incrementally from coast to coast, addressing problems of national interest.

A wide range of background research is essential for recombinant DNA based Biotechnology to flourish. We have been fortunate in having an excellent team providing the background information and know how for genetic engineering. We have tried to capture the spirit of this multi disciplinary team by listing the success stories of the various team members. Biotechnology takes its roots from several other studies that provide the necessary background information and support. Some of these teams are included to highlight their importance to our success.

This brochure is projected towards forest managers interested in the future of our forests, forest industries searching for innovative pest control that is sensitive to the environment, pesticide companies looking for target-specific designer biocontrol agents, researchers in biotechnology, biotech entrepreneurs interested in spin-off inventions, and the inquisitive public at large. We do not wish our research effort to be known as one of the "best kept secrets in the Canadian Forest Service"! We solicit criticisms and suggestions to enable us to improve the next edition of this brochure as well as our research.

Creation of this brochure has been a collective effort of the members of the Biotechnology Project. The contributions of Mark Primavera for the graphics, Bill Tomkins for the photographs, Marilyn Scott for word processing and Karen Jamieson for the editorial help are gratefully acknowledged. This brochure was made possible by a grant from the National Biotechnology Strategy Fund. A french version of this brochure should be available in the not too distant future. Credit for excellence belongs to the contributors and blame for omissions and oversight rests solely with me.

Arthur Retnakaran  
March 15, 1998

### Cover Photographs

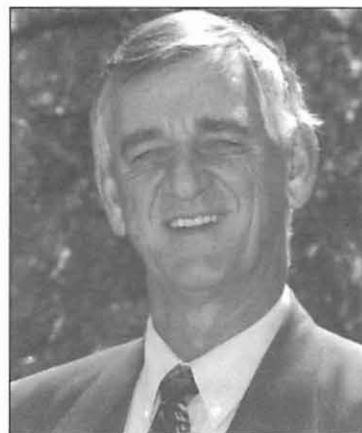
**Green fluorescent 6<sup>th</sup> instar spruce budworm:** This larva ingested a genetically engineered spruce budworm, *Choristoneura fumiferana*, nucleopolyhedrovirus (CfMNPV) expressing a green fluorescent protein gene (gfp) from a jelly fish which is widely used as a reporter gene. When the virus multiplies in the different tissues of the larva, the gfp gene is expressed and as a result the entire insect fluoresces when viewed under a fluorescence microscope.

**Electron micrograph of a cell showing polyhedra containing virions:** Section of a tissue cultured cell (IPRI-MD-66 cell line) infected with the forest tent caterpillar, *Malacosoma disstria*, nucleopolyhedrovirus (MdMNPV) showing a quadrant of the nucleus containing polyhedra (black bodies) containing virions.

**Effects of a transgenic spruce budworm virus expressing a molt regulatory gene cloned from the spruce budworm:** These 6<sup>th</sup> instar spruce budworm larvae were fed a genetically engineered virus (CfMNPV) expressing a molt regulating transcription factor (CHR3). Upon ingestion, the virus multiplies and over expresses the CHR3 gene and as a result the larva stops feeding and goes into an incomplete, precocious, molt that is lethal. Further work is currently underway.

## Foreword

The Canadian Forest Service mission is to promote the sustainable development of Canada's forests and the competitiveness of the Canadian Forest Sector for the well-being of present and future generations of Canadians. To fulfill this mandate, the CFS plays a strategic role for Canada in S&T research and in national policy coordination. On the research side, the CFS is the largest single organization in the country dealing in forest biotechnology with activities in the Tree Biotechnology and Advanced Genetics network and in the Pest Management Methods Network. On the policy coordination side, the CFS is involved in the renewal of the Canadian Biotechnology Strategy, which was initiated in 1983.



Forest biotechnology provides new and alternative tools that, in the context of sound forest management practices, could play a pivotal role in environmentally-friendly pest management. Almost 30 million acres of Canada's forests are subject to moderate to severe defoliation by insects each year and a proportion of this area could benefit from biopesticide treatment to increase forest productivity. Already, *Bacillus thuringiensis* (also known as *B.t.*) is an important management tool against the spruce budworm and its efficacy is being improved. New developments in the area of viral biopesticides are preparing the way for the second and third generations of biopesticides with increased specificity and efficacy.

In a larger context, biotechnology provides enabling technologies that could have a profound positive impact on Canada's economy and environment. For this reason, the federal government has identified biotechnology as a key component to build a competitive knowledge-based economy. In collaboration with other federal departments and agencies with an interest in biotechnology, the CFS is contributing to the renewal of the Canadian Biotechnology Strategy. This strategy will provide a framework to ensure the future development of biotechnology, fully taking into account its social, ethical, environmental and health aspects. It will also provide the means for efficient and informed public input.

The Canadian Forest Service is a leader in the development and the application of biotechnology for the forest sector. This has been accomplished through solid fundamental research linked closely to real problems for our forests. It has achieved several breakthroughs such as the development and registration of natural viral biopesticides, and the establishment of laboratory microcosms to evaluate the environmental safety of the newly developed biopesticides. The CFS is working for the future through its research on genetic engineering of trees and of biopesticides. In all cases, the breakthroughs and advances of our scientists were made in collaboration with other research organizations, universities and/or private industry. Partnership is instrumental to provide the resources and the focus for efficient science delivery. It is also essential for the commercial use of the biotechnology tools developed by CFS.

This document is intended as a point of entry into CFS expertise, science and technology. Our organization places high value on effective interactions with our partners. I invite you to actively pursue collaboration with CFS' scientists or to explore the commercial potential of our technologies.

**Yvan Hardy**

Assistant Deputy Minister  
Canadian Forest Service

**PROJECT TITLE:** Science Marketing

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**OBJECTIVE:** To effectively coordinate and market the expertise, science and science products of the Canadian Forest Service through memoranda of understanding, collaborative agreements, intellectual property protection, and licensing agreements.

**DESCRIPTION:** There is an increased emphasis in the Canadian federal government for better recognition of its activities and for increased external revenue generation. To this end, better coordination of marketing activities between the five CFS centres and the Science Branch is required. An internal working group has been formed to address this issue. Furthermore, science marketing is an emphasis of the federal government as a whole and the Science Branch marketing unit links the CFS with resources and expertise in other federal departments and agencies. Of particular interest is the Federal Partners in Technology Transfer which is a group providing support to all federal departments. The Science Branch marketing group is involved in the production of marketing material for the CFS looking at horizontal issues such as Climate Change, Biodiversity, Knowledge Initiative and, Criteria and Indicators. The group is also facilitating international marketing by pro-actively developing memoranda of understanding with different countries such as France and the United Kingdom for biotechnology. The marketing unit plays an active role in prospecting for and in facilitating interactions with industrial partners in all intellectual property issues.

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## PEST MANAGEMENT BIOTECHNOLOGY

### **Introduction**

The term “biotechnology” was coined in 1919 by Karl Ereky, a Hungarian engineer. At that time, the term meant all the lines of work by which products are produced from raw materials with the aid of living organisms. Biotechnology, in the present day, is the use of whole or parts of organisms, such as cells, genetic materials or enzymes to make or modify products, to improve valuable traits in plants or animals, or to develop organisms for specific applications. More recently, “Genetic Engineering”, a subdiscipline of biotechnology, has become predominant because it enables the introduction of desirable characteristics in existing species more rapidly and precisely than has previously been possible. For instance, it is possible to cross the species barrier and introduce genes from a eukaryote into a prokaryote and vice versa (e.g., human insulin gene expressed in bacteria and yeast). Biotechnology utilizes the varied capabilities of living organisms and therefore relies on biodiversity.

### **Genetic Engineering**

The Danish biologist, William Johanssen, devised the term “gene” in 1911 to distinguish “genotypes” from “phenotypes”. It was only three decades later, in 1944, that Oswald Avery and his colleagues at the Rockefeller Institute demonstrated that genes are made of DNA. One of the cornerstones in the development of genetic engineering occurred in 1953 when James Watson and Francis Crick explained the double-helix structure of DNA, which was followed by the deciphering of the genetic code. Twenty years later, Herbert Boyer and Stanley Cohen applied for a patent on techniques for recombinant-DNA technology and Boyer later became the co-founder of the first genetic engineering company, Genentech Inc. In 1980 when the US Supreme Court ruled that Chakrabarty’s engineered bacterium (which breaks down oil slicks) could be patented, Genentech’s stocks set a Wall Street record! Genentech’s bacterially produced human insulin was approved for use in the US in 1982. Subsequently, in 1985, the US declared that genetically engineered plants are patentable. While genetic engineering has suffered setbacks in Europe, the largest market in the world, the US, has become the bastion of recombinant DNA based biotechnology. To date, 24 transgenic plants, 11 transgenic animals and numerous engineered microorganisms have been patented. Biotechnology companies have mushroomed all over North America and their stocks (listed in NASDAQ) have performed extremely well.

### **Branches of Genetic Engineering**

The fact that a coding region of a gene can be taken from the DNA of one organism and transferred into another makes it possible to introduce a vast array of beneficial characteristics into life forms that are important to humans in terms of food, health, shelter, environment and quality of life. Methods of genetic engineering have been applied to almost every conceivable situation covering almost all disciplines. The major thrust, which has found widespread acceptance, is in the medical field. Synthesis of human hormones in microorganisms, development of vaccines, genetic medicine and gene testing, are some of the major achievements of the 20<sup>th</sup> Century. Agriculture is the second biggest area where genetic engineering has made significant inroads. Transgenic crops with superior yields, pest or herbicide resistance and cold tolerance are a few of the accomplishments. Environmental science has seen the utilization of engineered organisms to detoxify many types of poisonous materials from lakes and soil.

The pest control industry around the world is worth approximately 30 billion dollars. Herbicides, insecticides and fungicides are the three major pest control groups and account for 47, 29 and 19% of the market, respectively. Western Europe, the US and Japan account for nearly 70% of the worldwide sales.

Chemical control is being progressively replaced by biological control methods that offer environmental protection. The virulence of naturally occurring and host specific biological control agents can be enhanced by introducing the required desirable characteristics. Improving natural biocontrol agents wherever possible by genetic engineering is the hallmark of pest control biotechnology and numerous pest control companies have aggressive programs in this field.

### **Pest Management in Forestry**

Pest management strategies in forestry have targeted three major groups of pests: pathogenic microorganisms, competing vegetation and insect pests. Historically, they have been managed by chemical fungicides, herbicides and pesticides respectively. The deleterious effects of many of these chemical control agents on non-target species in the environment has resulted in the exploration of environmentally friendly, alternative methods.

Parasites, predators and entomopathogenic microorganisms have been used as environmentally safe biological control agents against forest insect pests with various degrees of success. To combat microorganisms causing tree diseases, forest managers have historically resorted to selecting resistant varieties of trees. A few species of fungi have been used for controlling weeds. With the advent of Biotechnology, there are various other options available for protecting trees against not only insect pests but also competing vegetation and organisms that cause tree diseases.

### **Pest Management Biotechnology**

In the broader sense, selection of resistant varieties of trees and using biorational control agents would be included under Biotechnology. But if one were to examine it from the subdiscipline of Genetic Engineering, a myriad of possibilities emerge. Fundamentally, it involves improving the biocontrol agents by using recombinant DNA technology. For instance, we could enhance the pest control activity by introducing genes from other species whose products are deleterious to the insect pest into a host-specific baculovirus. Use of genes from diverse sources of life forms is an integral part of biotechnology. The most common example is the use of the thermostable DNA polymerase for the polymerase chain reaction (PCR) which was obtained from the bacterium present in the hot springs of Yellowstone National Park. The green fluorescent protein gene (GFP) that is used widely as a marker was taken from a jelly fish. It has been aptly said that biodiversity sustains biotechnology since many of the donor genes are obtained from a wide variety of species.

In the Canadian Forest Service there are extensive opportunities in biotechnological research aimed at developing environmentally friendly alternatives to broad-spectrum chemical pesticides. Some of the more important ones are:

1. Recombinant baculoviruses for spruce budworm control;
2. Genetic engineering of mycopathogens for insect pest control;
3. Molecular characterization of viral, bacterial and fungal pathogens of trees - prelude to resistance studies;
4. Mechanisms of resistance to pathogens (virus, bacteria, fungi and nematodes) and insect pests as well as isolation of genes responsible for such resistance (transfer of technology to Tree Biotechnology network for insertion into trees);
5. Molecular biology of microsporidia and entomopathogenic nematodes - develop methods to enhance activity.

In all these investigations, knowledge at the molecular level of the pest organism, its host and their interaction have to be elucidated to develop a rational approach to increase the desired activity. With the personnel available it is not possible to cover all the areas to the same depth as one would like to. Therefore, without sacrificing the scope of our investigations, research areas have been prioritized. In addition, an infrastructure covering the supporting areas of research is essential.

### **Interaction with other Networks**

The research disciplines in the Canadian Forest Service have been collated under 10 networks across the country. Such a system will not only address the national needs within the limited resources, but also generate more knowledge than the sum of its components.

Pest management biotechnology is included as a part of the **Pest Management Methods network** and therefore a majority of the activity of this group is devoted to improving biological control agents for controlling forest pests. The molecular biology expertise available within this group can contribute significantly to many of the objectives of other CFS networks. For instance, information on the distribution of genetic material is essential for practising sustainable forest management to conserve biodiversity. Molecular techniques such as microsatellite analysis and polymerase chain reaction based DNA fingerprinting techniques are currently being used to address questions of genetic diversity. The Pest Management Biotechnology Group has state-of-the-art molecular biology facilities, including personnel, to perform such analyses for the **Biodiversity network**.

The interaction of forest trees, animals and microorganisms and the disturbance caused by harvesting, fires, pest damage, and intervention is one of the many facets addressed by the **Forest Ecosystem Processes network**. By developing genetic probes to type (unambiguously identify) varieties and strains of different species of trees, animals and microorganisms within the forest ecosystem, we will provide the backdrop for studying the effects of external disturbances on the forest ecosystem. The Pest Management Biotechnology Group can also contribute to a better and more comprehensive understanding of the life cycles of many species and the environmental factors that influence insect population dynamics. We have already identified genes that are expressed exclusively during the overwintering diapause of the spruce budworm; these can be used as invaluable tools to monitor the survival of the spruce budworm over the long winter period. We are also involved in studying the hormonal and other physiological control of insect life cycles, which is also addressed by this network.

One of the major objectives of the **Forest Health Network** is to establish a national health monitoring system that enables assessment and prediction of forest health. The molecular biology kits that are being developed by members of the Pest Management Biotechnology Group can be used to identify and monitor the disease causing pathogenic organisms as well as damaging pest populations.

Some of the basic research on the biology, physiology, biochemistry and molecular biology of key forest pests as well as host-parasite interaction studies will provide the data base for developing computer-based models and information technologies needed to manage forest landscapes under **Landscape Management Network**.

Recombinant DNA technology, like many new technologies, faces the challenge of social acceptability. Risk assessment and knowledge sharing to alleviate fear of the unknown are integral aspects of Pest Management Biotechnology. This work straddles the dual issues of economic viability and social acceptability and becomes a part of the **Socio-Economic Research Network**.

Construction of predictive models to aid forest managers to understand and cope with the impact of climate change on forest ecosystem, fire regimes and pest infestations are some of the major objectives of the **Climate Change Network**. Indicator genes of climate change can be cloned and genetic probes can be developed to study the effects of climate change on pest populations.

Development of economically feasible and socially responsible alternative practices that protect the ecosystem is one of the major objectives of the **Effects of Forestry Practices Network**. The Pest Management Biotechnology Group is involved in developing transgenic biological control agents that are not only effective but also target specific. The use of such control measures which will protect the environment, will directly influence the activities of this network. These products will be passed on to the members of the Effects of Forestry Practices Network to evaluate the economic feasibility and acceptance of these control agents.

The aim of the **Tree Biotechnology and Advanced Genetics Network** is to produce not only trees that are genetically superior but are also resistant to pests. The genes that are identified by the Pest Management Biotechnology Group to be toxic to forest insect pests and pathogenic microorganisms of trees will be passed on to the members of the Tree Biotechnology and Advanced Genetics Network for insertion into trees. The recombinant DNA technology used by the Tree Biotechnology and Advanced Genetics Network and our group is similar in most respects. Therefore there will be in the future, as it has been in the past, frequent exchange of both information and materials between the two networks.

**STUDY TITLE:** Molecular Virology

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVE:** To produce environmentally benign viral control agents against forest insect pests by studying the molecular virology of entomopathogenic viruses.

**DESCRIPTION:** Insect viruses present a potentially viable alternative to broad spectrum chemicals in the control of forest and agricultural insect pests. While they are environmentally attractive, insect viruses are generally host specific and slow acting. Research on insect viruses over the last few years has resulted in the characterization of the mode of action at the natural host and the cell lines levels. It became apparent that baculoviruses have genes that are not essential for replication and could be replaced by exogenous genes. This has resulted in the development of successful expression vector systems as well as the generation of biological control agents with enhanced effect on the target insect. The spruce budworm, *Choristoneura fumiferana*, is one of the most devastating pests, destroying millions of hectares of the forests annually. Over the last few years, efforts have been concentrated on the characterization of the genome and engineering of the baculovirus CfMNPV, originally isolated from the spruce budworm. A number of genes have been cloned, sequenced and characterized, which allowed the construction of vectors to transfer foreign genes to the virus. A number of engineered viruses have been generated, some of which appear to have enhanced activity against the spruce budworm. Also, a defective baculovirus (CfDEFNPV) found in wild-type preparations in the spruce budworm has been characterized and its relationship to CfMNPV has been elucidated. The defective virus requires CfMNPV as a helper to negotiate the gut barrier. However, while in susceptible larval tissues, CfDEFNPV synergizes the replication of CfMNPV. The defective virus is being developed as a self-limiting biological control agent against the spruce budworm.

Entomopoxviruses (EPVs) have morphological and structural similarities to vertebrate poxviruses but are distinguished by being occluded in a proteinic matrix at the end of the replication cycle. Two viruses (CfEPV, CbEPV) from the budworm species are being characterized at the molecular and the biological levels. Because of their morphological similarities to orthopoxviruses, EPVs may not be used as biological control agents at the present time. Nevertheless they contain genes that appear to enhance baculoviruses against the natural host. Research on CfEPV and CbEPV has been centered on the elucidation of the genome organization, characterization of viral genes and comparison of these genes to their vertebrate poxvirus homologues. Of a particular interest is the fusolin protein and its homologue encoded by the gp37 gene in baculoviruses. Phylogeny analysis indicates that the fusolin and gp37 are ancient genes and both proteins have diverged from a common ancestor and appear to be evolving at the same rate.



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**STUDY TITLE:** Disruption of insect metamorphosis by a polydnavirus

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVES:** To identify the mechanism(s) responsible for the disruption of spruce budworm metamorphosis following infection by a polydnavirus and to develop novel biorational control agents whose mode of action targets one of the identified mechanisms.

**DESCRIPTION:** This study was initiated in 1992 as part of the Green Plan's Forest Pest Management Initiative. The endoparasitic wasp *Tranosema rostrale* transmits a polydnavirus (PDV) to its spruce budworm host during parasitization. This virus depresses the budworm's immune system and fatally disrupts its development, to the benefit of the parasitoid. Although these viruses could not be used directly as viral insecticides, we believe that the mechanism(s) responsible for the observed virally induced pathologies could be exploited in the development of biorational control agents. So far, we have characterized the effects of this virus on the immunity, development and hormonal balance of spruce budworm larvae. We are currently studying PDV-induced disruptions at the molecular level in an attempt to identify relevant viral genes. These viruses also serve as tools to study the hormonal and molecular events leading to metamorphosis. This study involves the collaboration of scientists from CFS/Atlantic, Dalhousie University, the University of Guelph and Laval University. Two graduate students have also contributed to this research.

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**STUDY TITLE:** Molecular monitoring of forest pathogens

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVE:** To develop novel ways to diagnose and detect forest pathogens in order to prevent introduction of new exotic pests or to prevent further spread of current epidemics.

**DESCRIPTION:** Many important forest diseases are caused by exotic pathogens that were introduced accidentally to this continent. These diseases, once introduced, are very difficult to control since they have no natural enemies and their hosts have little or no resistance. One of the problems with many forest pathogens is that they can remain latent for periods as long as several years. Therefore, monitoring and health certification can be difficult or inefficient. We have developed a method based on DNA amplification techniques (U. S. patent pending) that allows us to detect root rots and scleroderris canker directly from infected seedlings even in the absence of symptoms, without having to culture the fungi. Therefore, a process that used to take weeks or months can now be done within a day. We are also currently involved in the development of new DNA probes that will allow us not only to type seedlings for the presence of a fungal pathogen, such as scleroderris canker but also to determine whether they are infected by a more virulent race, such as the exotic European race or by a the less virulent race, such as the native North American race. We are also planning the development of "user-friendly" diagnostic tools, such as colorimetric assay kits, that would be usable by field technicians without the need for molecular expertise. This research involves several collaborators from Universities and Biotechnology companies

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**STUDY TITLE:** Molecular Entomology

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVE:** To study the molecular mechanisms of molting and metamorphosis of forest insect pests and use the information for developing environmentally benign pest control agents.

**DESCRIPTION:** Molting is a critical phenomenon in insects. Interfering with any of the regulatory mechanisms involved in this process can result in the death of the insect. We embarked on a study of the molting process at the molecular level and so far we have successfully cloned and characterized cDNAs for ecdysone receptor (CfEcR), ultraspiracle (Cfusp), *Choristoneura* hormone receptor 3 (CHR3), *Choristoneura* hormone receptor 75 (CHR75) and Juvenile hormone esterase (JHE). We have tested the effects of over or under expressing some of these genes using *Choristoneura fumiferana* nucleopolyhedrovirus (CfMNPV). Overexpression of CHR3 induced precocious molting and the larvae remained moribund in this state (see cover picture). We are also working on the promoters of genes such as CHR3 and CHR75 with a view to developing high throughput assays for screening new ecdysone mimics. Juvenile hormone (JH) is a unique hormone in insects that plays an important role in growth, metamorphosis and reproduction. In order to understand the action of JH at the molecular level, a differential display of mRNAs technique was used to clone JHE from CF-203 cells that were grown in the presence of juvenile hormone I (JHI). This gene is directly induced by JHI and is suppressed by 20-hydroxyecdysone and is the first example of dual regulation by these two hormones. We plan to use its promoter to identify the JH receptor and develop high throughput assays to screen for more potent JH mimics.

The ecological adaptation of overwintering is a critical phenomenon in the life of an insect. The spruce budworm overwinters as a diapausing second instar larva and this diapause is obligatory. We have identified two major proteins associated with diapause, and using the differential display of mRNAs technique we have cloned the first lepidopteran defensin gene from insects. The defensin protein is active against both gram positive and negative bacteria.

In an attempt to understand the molecular basis of host specificity of baculoviruses, we are investigating apoptosis or programmed cell death in CF-203 cells induced by such viruses. We have discovered that certain baculovirus genes can block this process. We are also studying the infection process of baculoviruses in larvae or *in vivo* using the jelly fish green fluorescence protein gene engineered into baculoviruses (see cover).

Our molecular studies can significantly contribute to other networks as well in understanding some of the ecological and physiological interactions. For example we plan to develop molecular detection kits as well as perform DNA finger printing of parasites and predators of forest defoliators for the Biocontrol group. We can use these techniques for studying competition among microorganisms for the microbial control group. We plan to conduct molecular analysis of genetic diversity of spruce budworm populations for the Biodiversity network. Using temperature and photoperiod sensitive genes we hope to study the effects of climate change on forest insects. We also plan on investigating the effect of environmental factors on the diapause phenomenon at the molecular level for the Ecosystem Processes network.

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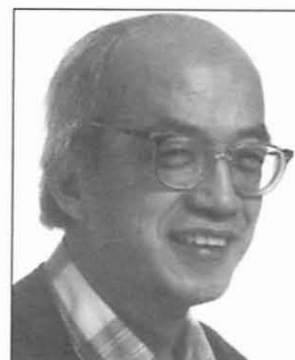
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**STUDY TITLE:** Immunochemistry

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVE:** To apply immunochemical technologies to study the identification, detection and mode of action of insect pathogens and to understand the defence mechanism of insects.

**DESCRIPTION:** This study was originally focussed on the serotyping of different *Bacillus thuringiensis* strains, and the mode of action of *Bacillus thuringiensis* delta-endotoxin. With the advanced development of immunochemistry and biotechnology, this study will be rapidly enlarging to cover other areas of research. Currently, the following studies are being conducted: (1) develop simple immunoassays for the detection of microbial pathogens of the spruce budworm, especially the spruce budworm virus, CfMNPV; (2) use immunochemical techniques for characterizing baculoviruses and spruce budworm specific proteins; (3) study the immuno-defence mechanism in the spruce budworm; and, (4) further investigation of the mode of action of *Bacillus thuringiensis* delta-endotoxin. This last study is in collaboration with the research scientists in the Microbial Pathogens and Environmental Impact groups within the CFS, and with the Department of Microbiology and Immunology at Queen's University, where the study leader holds an adjunct position.

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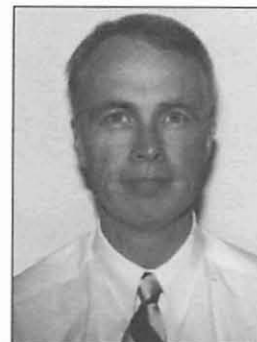


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**STUDY TITLE:** Virus Ecology

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVE:** To investigate the ecology and mass propagation of naturally-occurring and genetically engineered insect viruses with a view to developing these agents into operational insect pest controls.

**DESCRIPTION:** This study was initiated in 1981 to investigate the relationship between insect viruses and their hosts in the environment. This research involves determining the response of the host insect to the virus infection, developing techniques to indicate the presence of the virus in insects and the environment, and investigating the interactions between insect viruses, their hosts and the environment. In the course of our research various larval bioassay techniques have been developed to quantify host response, and a cold probing technique was developed for the detection of baculoviruses in the environment. More recently, the study of mass propagation of viruses for operational use was initiated, and a pilot production plant set-up. Study activity has focused mainly on baculoviruses of the spruce budworm, gypsy moth and forest tent caterpillar. Various commercial and governmental organisations have been involved in collaborating with or funding these activities. American Cyanamid collaborated in the development of an NPV product for gypsy moth control, and in-vivo production of Phospholipase A<sub>2</sub> for pharmacological research was undertaken in collaboration with Merck Frosst, using genetically modified *Autographica californica* NPV in *Trichoplusia ni* larvae. In recent years study activity has concentrated on the bioassaying of genetically engineered baculoviruses using new types of bioassay appropriate for quantifying GEMS, with sponsorship from the National Biotechnology Strategy fund, and pursuing the use of a new spruce budworm baculovirus isolate (T3) that shows promise for operational application, in collaboration with BioSag, a private Quebec company.

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**STUDY TITLE:** Insect Physiology

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVE:** To study the physiology of growth, development, reproduction and metamorphosis of forest insect pests for the rational use of Insect Growth Regulators, Biorational control agents and genetically engineered biological control agents for managing insect pests of forests.

**DESCRIPTION:** The unique physiological processes that are seen in insects can be exploited for controlling insect pests without adversely affecting the environment. With such a goal in mind, this study was initiated to investigate the effects of hormone analogs, chitin synthesis inhibitors and insect growth regulators on several forest insect pests. Fundamental studies on the physiology of the epidermis, chitin formation and hormonal control of metamorphosis were carried out as a backdrop to the research on insect growth regulators. Cooperative work with industries such as Philips-Duphar (Holland), Bayer (Germany), Hoechst (Germany), Ishihara (Japan), Sumitomo (Japan), Eli Lilly (USA), Zoecon (USA), Chemagro (USA), Ayerst (Canada), Ciba-Geigy (Switzerland), Dr. Maag (Switzerland), Imperial Chemicals (U.K.), Rohm and Haas (USA & Canada), and Dow Chemicals (USA), for various periods of time have provided the industrial support for the applied research and field trials carried out in various parts of Canada. A few of the memorable trials were, juvenile hormone analogs against the hemlock looper in Anticosti island in collaboration with Dr. Luc Jobin, Dimilin against the hemlock looper in Bay Despoir, Newfoundland with Dr. Rick West and RH-5992 against the spruce budworm in Longlac with Mr. Leo Cadogan. We have also demonstrated in the field that the white pine weevil damage can be effectively controlled by spraying Dimilin on tree shoots prior to the emergence of weevils in early spring. When the weevils feed on the contaminated shoots they invariably lay sterile eggs. More recently, in collaboration with other CFS colleagues, the molecular mode of action of RH-5992, a nonsteroidal ecdysone agonist was elucidated. The mode of action and expression of selected genes using recombinant baculoviruses are currently being studied using, among other techniques, *in situ* hybridization. We have recently shown that the first instar spruce budworm feeds by grazing on the surface of the needles, a finding that eluded researchers for nearly five decades. This exciting discovery opens up a whole new approach to controlling this devastating pest before it has a chance to cause any damage.

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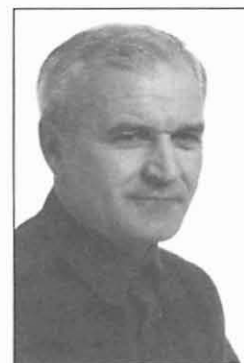
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**STUDY TITLE:** Molecular Forest Pathology

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVE:** To study the biology and genetics of phytopathogenic fungi for the rational use of spore deficient mutants, biological control agents and genetically engineered plants.

**DESCRIPTION:** Major diseases affecting our forests are caused by highly virulent sporulating fungal pathogens. For the development of new and innovative biological methods for forest disease control the identification of genes or gene products responsible for blocking/regulating a pathogen's capability to sporulate would prove beneficial. This would be a major breakthrough in forest pathology research. Incorporation of this genetic information into the host, alternate host, or various endo/epiphytes for expression purposes would reduce the need for routine chemical application and would introduce to the environment a host-compatible biological control system with patent potential and great public support. It would also serve as technology transfer for future developments in plant breeding strategies (forest and/or agriculture) against fungal pathogens in which disease spread is dependent on the pathogens capability to produce a spore phase in their life cycle.

This study was initiated in 1993, based on this novel strategy for tree disease control. The organism chosen for investigation was *Ophiostoma ulmi*, the causal fungus of one of the most devastating tree diseases known to mankind, vascular wilt of elm. The isolation of novel spore deficient mutants for the three major spore types of *O. ulmi*: 1) a conidiospore deficient mutant for which the spore deficient character has been linked to a single nuclear gene, 2) an ascospore deficient mutant and 3) a blastospore deficient mutant has contributed significantly to the advancement of this study. Genes or gene products that contribute to the blocking/regulation of these various spore forms will provide specific information upon which to develop new biological methods for disease control.

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**Richards W. C.** 1997. Novel spore deficient mutants of the dimorphic fungal plant pathogen, *Ophiostoma ulmi*. Crop Health Unlimited Horizons. CPS/APS joint meeting, July 6-7/97. Winnipeg Manitoba. Phytopathology (in press).

**STUDY TITLE:** In situ hybridization

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVES:** To detect in time course studies the presence of certain pathogens or to reveal the presence of transcripts of some genes using in situ hybridization techniques.

**DESCRIPTION:** As a model system, we are currently working to produce different probes with the best labels possible for the detection of the tobacco mosaic virus (TMV) and the tomato mosaic virus (ToMV) in heavily infected tobacco leaves. Thereafter, we want to use these probes to detect TMV in ash showing symptoms of viral infection in the province of Quebec and/or ToMV in conifers. Also, we want to work on two other important tree diseases: the scleroderris canker of conifers and the white pine blister rust. Under favorable conditions, these diseases may have devastating effects on some of our most valuable timber species. Even if these commercial losses have stimulated many types of investigations, the knowledge still remains fragmentary and many other studies should be conducted to devise better control measures of these diseases. In particular, many aspects of the mode of ingress and development in the host are to be investigated. In this respect, *in situ* hybridization methods might for instance permit researchers to follow the path of a few scattered pathogen cells from the start of the infection in needles to their establishment in larger parts of the tree where they induce canker development. These methods might also enable us to distinguish the targetted pathogen cells from others that might also be present in host tissues, such as endophytic fungal cells. Likewise, in the forest, to study the mode of spread of pathogens, it might be possible to collect spores dispersed in the air and, following amplification of the desired portion of nucleic acids by *in situ* polymerase chain reaction (PCR), to determine the genotype of the pathogenic agent by *in situ* hybridization techniques. Thus, it might be possible to learn the origin of these spores since the genotype of many cankers has already been established in several sampling units. These data thereafter might be used to predict the course of the epidemic.

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**STUDY TITLE:** Insect Tissue Culture

**PROJECT TITLE:** Pest Management Biotechnology

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**OBJECTIVE:** To develop and apply insect tissue culture systems for the *in vitro* investigations of insect control agents for developing environmentally benign and effective biorational insecticides for forestry use.

**DESCRIPTION:** With the recent advances in biotechnology and genetic engineering, insect tissue cultures have assumed an increasingly prominent role in basic biological research and biotechnology during the last decade. They are now routinely used in a wide variety of biomedical studies. Insect cell cultures are essential for investigations of most insect pathogens, and their use in other entomological research is increasing every year. They are critical for the genetic engineering and strain selection of insect viruses and are also increasingly being used for the strain selection of *Bacillus thuringiensis* (*Bt*) and for the *in vitro* bioassay of insect viruses and *Bt* toxins. Besides, insect cell cultures are required for producing foreign gene products using baculovirus expression vector systems. Moreover, insect cell cultures will be crucial for the mass production of insect pathogenic viruses for use in the integrated pest management, because they offer a cleaner, viable, and probably in the long run a cheaper alternative to insect larvae for producing viral pesticides.

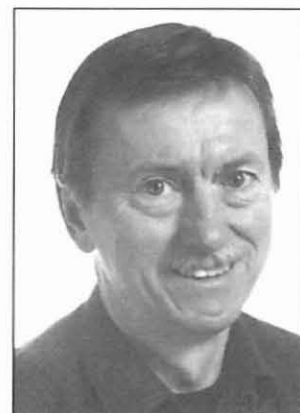
We have developed over 80 continuous cell lines from the tissues of the spruce budworm, forest tent caterpillar, tobacco hornworm, white-marked tussock moth, red-headed pine sawfly and the white pine weevil. Some of these cells have been in culture since 1969, and in collaboration with other CFS colleagues we have demonstrated that several of our cell lines support the replication of several insect viruses and microsporidia. Also, we have developed a cell culture bioassay for *Bt*  $\delta$ -endotoxins and  $\beta$ -exotoxins, and have used several of the cell lines for studying the mode of action of these toxins. Some of our insect cell lines undergo apoptosis when exposed to the inhibitors of RNA- and protein-synthesis, and to certain viruses. Furthermore, several of these cell lines respond to the molting hormone ecdysone and its nonsteroidal agonists, RH-5849 and RH-5992, and to the juvenile hormone. Currently, we are screening our cell lines for the replication of more insect viruses and are cloning some of the lines for improving virus replication and yield. Also, we are working on developing the methodology for growing large-scale cultures of insect cells for virus production in collaboration with the Biotechnology Research Institute, Montreal.

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**STUDY TITLE:** Electron Microscopy

**PROJECT TITLE:** Research/Technical Services

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**OBJECTIVE:** To provide a timely and reliable range of high quality electron microscopy services to the scientific staff of Great Lakes Forestry Centre and networked collaborators as warranted. Services include transmission (TEM) and scanning (SEM) electron microscopy and specialised techniques such as immunogold labelling, negative staining, etc.

**DESCRIPTION:** This year marks the 50<sup>th</sup> anniversary of electron microscopy services at this research centre. Over the years the focus has changed from simple insect pathology to include all manner of forest pests and pathogens, complex physiology, cellular interactions, viral replication and infections, immune reactions, cell death and apoptosis implying considerable participation in biotechnology studies. With the addition of SEM facilities we have added the capacity to observe minute surface details of insects, fungal pathogens and their host plants which can assist in taxonomy studies, show the effects of toxins and growth regulators and expose mechanical damage.

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Baines, D., **Brownwright, A.J.** and Schwartz, J.L. 1994. Establishment of primary and continuous cultures of epithelial cells from larval lepidopteran midguts. J. Insect Physiol. 40 No.4: 347-357

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- Barrett, John W., **Brownwright, Andrew J.**, Primavera, Mark J. and Palli, Subba Reddy 1998. Studies of the nucleopolyhedrovirus infection process in insects by using the green fluorescence protein as a reporter. J. Virol.

**STUDY TITLE:** Environmental Toxicology of Forestry Pesticides

**PROJECT TITLE:** Environmental Research and Assessment

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**OBJECTIVE:** To study the environmental effects of forestry pesticides, in particular naturally occurring and genetically engineered microbial pest control agents.

**DESCRIPTION:** This study addresses concerns related to the environmental safety of forestry pesticides. Recent work has focused on the development of environmental testing protocols for naturally occurring and genetically engineered insect baculoviruses, including non-target bioassays, and forest soil and aquatic microcosms. Effects on individuals, populations, communities and processes are investigated. Methods are being developed to track viruses and viral DNA in insects and other organisms, soil, litter, water and aquatic sediments.

#### **PUBLICATIONS:**

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**STUDY TITLE:** Microbial Pathogens of Forest Pest Insects

**PROJECT TITLE:** Microbial Control Agents

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**OBJECTIVES:** To determine the impact of naturally occurring microbial pathogens on insect populations and the potential of specific fungi, protozoa and viruses for use in biological control through studies on their life cycles, cytology, genetics, epidemiology and methods for mass production.

**DESCRIPTION:** Forest pest insects, like all organisms, are preyed upon by other animals and suffer from a myriad of microbial diseases. Among these microbial pathogens are viruses, bacteria, protozoa and fungi. While all microbial pathogens are considered in our studies on disease incidence in insect populations, the focus for use in microbial control is on viruses, protozoa and fungi. Pest insects currently being studied are hemlock loopers (*Lambdina fiscellaria*), spruce budworm (*Choristoneura fumiferana*), whitemarked tussock moth (*Orgyia leucostigma*) and balsam fir sawfly (*Neodiprion abietis*). Our research has involved collaborative partnerships with clients and researchers from the provincial governments of New Brunswick, Newfoundland, Nova Scotia and Québec, Forest Protection Ltd., Institut Armand-Frappier, Dalhousie University and the Universities of Victoria, Maine and New Brunswick.

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**STUDY TITLE:** Research and Technical Services

**PROJECT TITLE:** Plant Production

**STUDY LEADER:** Mr. Scott Macleod  
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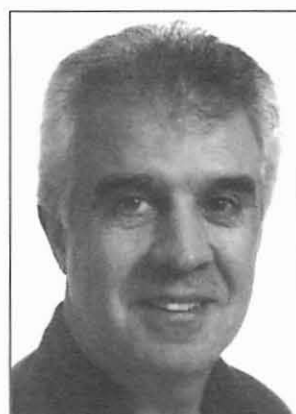
**OBJECTIVE:** To provide greenhouse space and a constant supply of plant material in support of forest research.

**DESCRIPTION:** This study provides a continuous supply of boreal foliage. Potted spruce, balsam, jack pine, white pine, and red pine are stocked. These trees are flushed year-round through use of environmental chambers and greenhouse facilities. White mulberry is produced continuously to feed and maintain a Silkworm culture. Ours is one of a handful of greenhouses in North America that maintains such a facility. Foliage is collected on a weekly basis locally, and seed collection of hardwoods is carried out seasonally. Greenhouse and controlled environment chambers are provided to synthesize biological conditions enabling forest pest controls to be applied and tested on site. Large numbers of coniferous tree seedlings of different ages are held in holding pens for use as needed. Some of these potted trees are forced to flush at desired times by photoperiodic, cold, and fertilizer treatments in walk-in cold rooms for testing pesticides to establish dosages for field trials. Greenhouse cubicles with individual photoperiodic and temperature controls for conducting experiments with potted trees are maintained throughout the year.

**STUDY TITLE:** Insect Production

**PROJECT TITLE:** Insect Production

**STUDY LEADER:** Mr. Bob McCron  
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**OBJECTIVE:** To produce and supply healthy insects. To improve the quality of all insect stocks and to establish rearing techniques for insects causing problems in all areas of Canada and modify existing rearing methods to fit our operation.

**DESCRIPTION:** Established in 1963, the Insect Production Unit has developed into the largest of its kind in Canada, supplying insects not only to staff of the Great Lakes Forestry Centre but also to various Natural Resources Canada establishments, universities and other research centres across Canada and the United States. The major demand from clients is for eastern spruce budworm, *Choristoneura fumiferana*. We produce approximately 3.5 million budworm annually; 1.3 million of these are supplied to outside clients.

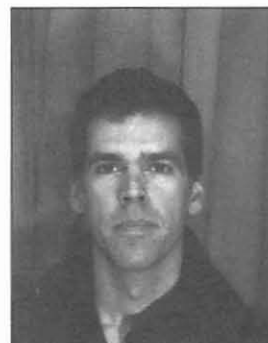
Over 4.5 million individual insects, representing fifteen species, are reared yearly. Research successes include the development of several non-diapause strains of insects, such as the western spruce budworm, *Choristoneura occidentalis*, and successful establishment of laboratory cultures of hemlock looper, *Lambdina fuscicollis fuscicollis*, and the fir coneworm, *Dioryctria abietivorella*. About 5,000 litres of artificial diet are prepared and dispensed annually to support the Insect Production Unit. These artificial diets are continually improved and modified to provide better insect growth and to establish cultures of new insect species.

Inventory of Insect Species In Culture (1998)	Origin of Culture
- <i>Actebia fennica</i> (Tauscher) Black Army Cutworm	Elliot Lake, Ontario
- <i>Bombyx mori</i> (Linnaeus) Silkworm (Nichi #102-bivoltine)	Experimental Sericulture Station, Tokyo, Japan
- <i>Choristoneura conflictana</i> (Walker) Large Aspen Tortrix	Several locations in Ontario
- <i>Choristoneura fumiferana</i> (Clemens) Eastern Spruce budworm (Also a non-diapause strain)	Several locations in Ontario
- <i>Choristoneura occidentalis</i> Freeman Western Spruce Budworm (Also a non-diapause strain)	Kamloops, B.C. Berkeley stock crossed with strain #COC-FS-01 from Corvallis, Oregon
- <i>Choristoneura pinus pinus</i> Freeman Jackpine Budworm	Webwood, Ontario
- <i>Dioryctria abietivorella</i> (Groté) Fir Coneworm	Thessalon, Ontario
- <i>Gilpinia hercyniae</i> (Hartig) European Spruce Sawfly	Manitoulin Island, Ontario
- <i>Lambdina fiscellaria fiscellaria</i> (Guenée) Hemlock Looper	Corner Brook, Newfoundland
- <i>Lymantria dispar</i> (Linnaeus) Gypsy Moth (Also a non-diapause strain)	Southern Ontario & Nova Scotia & Otis Air Base, USDA
- <i>Malacosoma disstria</i> Hübner Forest Tent Caterpillar	Several locations in Ontario
- <i>Orgyia antiqua</i> (Linnaeus) Rusty Tussock Moth	Minnesota, USA
- <i>Orgyia leucostigma</i> (J.E. Smith) Whitemarked Tussock Moth	Sault Ste. Marie, Ontario
- <i>Orgyia pseudotsugata</i> (McDunnough) Douglas-fir Tussock Moth	USDA- Virus Facility Corvallis, Oregon, USA
- <i>Trichoplusia ni</i> (Hübner) Cabbage Looper	Agriculture Canada Harrow, Ontario

**STUDY TITLE:** Genetic Engineering for Pest Tolerance

**PROJECT TITLE:** Tree Biotechnology

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**OBJECTIVE:** Our research aims to isolate and characterize genes from spruce and poplar which are involved in the molecular processes of stress-response, and identify the potential signals responsible for the induction of these responses.

**DESCRIPTION:** We have isolated a substantial number of cDNA clones, mainly using PCR technology, that show elevated expression after application of a specific stress (wounding or fungal elicitor). White spruce cDNAs showing homology to dehydrin, proteinase inhibitor, 14-3-3, peroxidase, ubiquitin, and a glycine-rich RNA binding protein have been obtained. Similar homologs have also been isolated from poplar. In most cases, this is the first evidence of the presence of these genes in forest trees. We are currently studying the expression of these genes to gain a better understanding of stress response mechanisms.

This research has many benefits. First, it will provide the fundamental knowledge that is essential for a better understanding of tree response after the attack of a pest. This information could be useful in understanding how a biological control method influences the physiological status of a tree. Secondly, the promoter of the characterizes stress-inducible gene could be used to obtain proper expression (e.g., wound-inducible) of a pest-resistance gene (e.g., the B.t. toxin). As mentioned earlier, this project will finally focus on the engineering of trees for improved silvicultural traits such as pathogen tolerance.

More applied aspects of my research focus on the introduction of the gene encoding the *Bacillus thuringiensis* toxin into *P. glauca* and the study of resistance of the transgenic plants to spruce budworm disease.

#### **PUBLICATIONS:**

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- Huang, X-F., Nguyen-Quoc, B. Li, Y-H., **Séguin, A.** and Yelle, S. (1997) Tissue-specific expression of two maize sucrose synthase promoters via particule bombardement. (in press, Euphytica ).
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## **A glimpse of Biotechnology infrastructure at the Great Lakes Forestry Centre in Sault Ste. Marie**

Biotechnology is heavily dependent on a variety of research facilities ranging from the simple to the sophisticated. In this first issue we hope to provide you with a tour of our more important work stations at the Great Lakes Forest Centre. In the next edition of this brochure we plan on covering the infrastructure at the other participating Canadian Forest Service research centres.

Since we are a nascent group, our research has not expanded into all the fields that we hope to ultimately address. Our first aim was to expand on our strength and therefore we embarked on a program to develop an environmentally friendly, target-specific transgenic virus for spruce budworm control, which will be as effective as or better than the control agents currently in use. The spruce budworm nucleopolyhedrovirus (CfMNPV) has been relatively well characterized in our research establishment and we decided to clone an insect gene into it to enhance its activity. We came up with several candidate genes cloned from the spruce budworm that we thought would enhance the activity of the virus. We succeeded in introducing the CHR3 gene, which is a transcription factor involved in the regulation of the molting process. This modified virus when ingested by the spruce budworm larva induces a precocious, incomplete molt that is lethal (see cover picture). It is many times more active than the wild type virus, is budworm specific and, since it has the gene from the host insect, does not introduce any exotic gene into the environment. We are in the process of increasing the activity of this transgenic virus by fine tuning the various factors that play a role in the over expression of this gene. Concomitantly, we are developing methods for mass producing the virus *in vivo* in spruce budworm larvae and *in vitro* in cell culture systems. Having succeeded in our primary objective we are confident that we will be able to expand our research into other areas of pest control.

The knowledge base required for the transgenic virus work covers a wide spectrum of research activity. Molecular virology, virus ecology, tissue culture, insect physiology, molecular entomology and immunology were the fundamental areas that contributed to the development of the genetically engineered virus. A great deal of fundamental research needs to be done to come up with a winning formula. The wealth of information that we have built during the past few years is outlined in the individual reports.

Success in research for the most part depends on the skills of the team members. In our description of the infrastructure in the succeeding few pages we will introduce the reader to some of the key players and their claim to fame.



**Peter Ebling (Virus Ecology Technician)** dispensing virus samples for bioassaying the insecticidal activity.

**Linda Sloane (Virus Ecology Technician)** injecting virus samples into spruce budworm larvae.

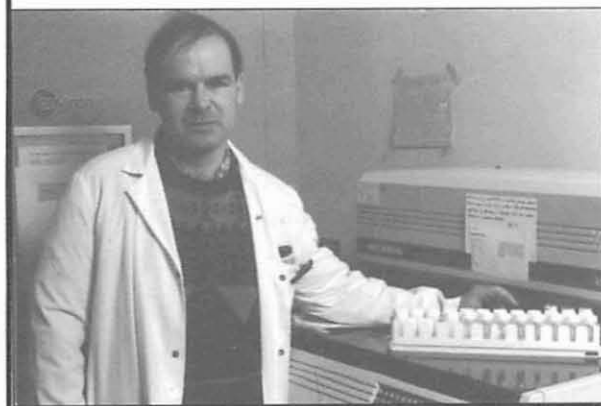


**Dr. Anthony Pang (Immunologist)** amplifying antibodies in a Harvest Mouse.



**Hilary Lauzon (Molecular Virology Technician)** getting ready to sequence a gel in an automatic sequencer.

**Dr. Li Xing (Post Doctoral fellow in Molecular Virology)** centrifuging samples in an ultracentrifuge.



**Peter Jamieson (Biologist in Molecular Virology)** counting radioactivity in a scintillation counter.



**Barb Cook (Tissue Culture Technician)** examining cells under the inverted microscope.

**Guido Caputo (Tissue Culture Technician)** isolating single cells for cell cloning in the QUIXELL cell transfer system.



**Mark Primavera (Insect Physiology Technician)** preparing tissue sections for *in situ* hybridization.



**Bill Tomkins (Insect Physiology Technician)** getting ready to examine slides in a fluorescence microscope.

**Tim Ladd (Molecular Entomology Technician)** preparing DNA primers in an oligosynthesizer.

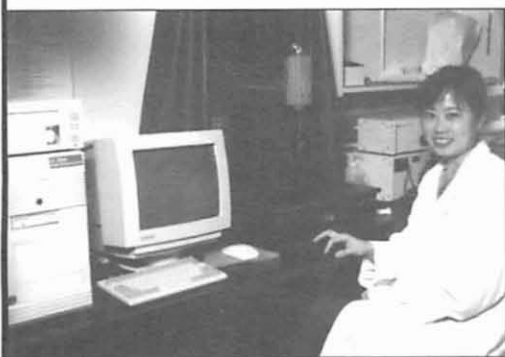


**Dr. Qili Feng (Post Doctoral fellow in Molecular Entomology)** examining bacterial colonies in a biohazard hood.



**Dr. Srimi Perera (Post Doctoral fellow in Molecular Entomology)** exposing dot blots in an instant imager.

**Dr. Meenakshi Sundaram (Post Doctoral fellow in Molecular Entomology)** examining Green Fluorescent Protein(GFP) labelled tissue under a GFP-microscope.



**Sichun Zheng (Molecular Entomology Technician)** viewing a gel in a photo documentation system.





**Gail Mick (Insect Rearing Technician)** collecting spruce budworm egg masses laid on the needles of balsam fir branches for rearing.

**Sharla Charrette (Insect Rearing Technician)** preparing artificial diet for rearing the spruce budworm.



**Suzanne Palmer (Insect Rearing Technician)** separating male and female spruce budworm pupae for setting up mating.



**Katriona Barratt (Insect Rearing Technician)** setting up silkworm larvae in cages and providing them with mulberry leaves grown in our greenhouse.



**Scott Macleod (Greenhouse Technician)** maintaining potted balsam fir seedlings for research in the greenhouse.



**Andy Brownwright (Electron Microscopy Technician)** viewing thin sections under the electron microscope.

# CFS S&T Networks

## Réseaux de S&T du SCF

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### **Forest Biodiversity Network / Réseau sur la biodiversité des forêts**

**Lead Centre/Centre directeur:** Atlantic Forestry Centre  
Fredericton, NB/ Centre de foresterie de l'Atlantique  
Fredericton (Nouveau-Brunswick)  
**Acting network manager/Gestionnaire du réseau intérimaire:** Bruce Pendrel  
Tel/Tél: 506-452-3500

### **Forest Health Network/Réseau sur la santé des forêts**

**Lead Centre/Centre directeur:** Atlantic Forestry Centre  
Fredericton, NB/ Centre de foresterie de l'Atlantique, Fredricton (Nouveau-Brunswick)  
**Network manager/Gestionnaire du réseau:** Thomas Sterner  
Tel/Tél: 506-452-3500

### **Climate Change Network/Réseau sur la changement climatique**

**Lead Centre/Centre directeur:** Northern Forestry Centre  
Edmonton, AB/ Centre de foresterie du Nord  
Edmonton (Alberta)  
**Network Manager/Gestionnaire du réseau:** Surj Malhotra  
Tel/Tél: 403-435-7210

### **Tree Biotechnology & Advanced**

**Genetics Network/Réseau sur la biotechnologie des arbres et la génétique de pointe**  
**Lead Centre/Centre directeur:** Laurentian Forestry Centre, Sainte-Foy, PQ/ Centre de foresterie des Laurentides  
Sainte-Foy (Québec)  
**Network Manager/Gestionnaire du réseau:** Ariane Plourde  
Tel/Tél: 418-648-5847

### **Fire Management Network/Réseau sur la gestion des feux de forêts**

**Lead Centre/Centre directeur:** Northern Forestry Centre  
Edmonton, AB/ Centre de foresterie du Nord  
Edmonton (Alberta)  
**Network Manager/Gestionnaire du réseau:** Denis Dubé  
Tel/Tél: 403-435-7210

### **Effects of Forestry Practices Network/Réseau sur l'aménagement des paysages**

**Lead Centre/Centre directeur:** Pacific Forestry Centre  
Victoria, BC/ Centre de foresterie du Pacifique, Victoria (Columbia-Britannique)  
**Network Manager/Gestionnaire du réseau:** Paul Addison  
Tel/Tél: 250-363-0600

### **Socio-economic Research Network/Réseau de la recherche socio-économique**

**Lead Centre/Centre directeur:** Northern Forestry Centre  
Edmonton, AB/ Centre de foresterie du Nord  
Edmonton (Alberta)  
**Network Manager/Gestionnaire du réseau:** Steve Price  
Tel/Tél: 403-435-7210

### **Pest Management Methods Network/Réseau sur les méthodes de lutte contre les ravageurs**

**Lead Centre/Centre directeur:** Great Lakes Forestry Centre  
Sault Ste. Marie, ON/ Centre de foresterie des Grands Lacs  
Sault Ste. Marie (Ontario)  
**Network Manager/Gestionnaire du réseau:** Errol Caldwell  
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### **Forest Ecosystem Processes Network/Réseau sur les processus des écosystèmes forestiers**

**Lead Centre/Centre directeur:** Laurentian Forestry Centre  
Sainte-Foy, PQ and Great Lakes Forestry Centre  
Sault Ste. Marie, ON/ Centre de foresterie des Laurentides, Sainte-Foy (Québec) et Centre de foresterie des Grands Lacs  
Sault Ste. Marie (Ontario)  
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### **Landscape Management Network/Réseau sur les incidences des pratiques forestières**

**Lead Centre/Centre directeur:** Pacific Forestry Centre  
Victoria, BC/ Centre de foresterie du Pacifique, Victoria (Columbia-Britannique)  
**Network Manager/Gestionnaire du réseau:** Murray Strome  
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# Canadian Forest Service Service Canadien des forêts

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## Service canadien des forêts

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## Canadian Forest Service Pacific Forestry Centre

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### Boyd Case

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## Canadian Forest Service Atlantic Forestry Centre

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## Service canadien des forêts Centre de foresterie de l'Atlantique

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## Canadian Forest Service Laurentian Forestry Centre

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