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# Genotypic variation and presence of rare genotypes among Douglas-fir tussock moth multicapsid nucleopolyhedrovirus (*Op*MNPV) isolates in British Columbia

Holly L. Williams, Imre S. Otvos *

*Natural Resources Canada, Canadian Forest Service Pacific Forestry Centre, Victoria, BC., Canada V8Z 1M5*

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**Abstract**

The Douglas-fir tussock moth (*Orgyia pseudotsugata*) multicapsid nucleopolyhedrovirus (*Op*MNPV) is periodically applied to suppress Douglas-fir tussock moth populations in British Columbia and in the western United States. The strain of *Op*MNPV in the product currently used for suppression is not genetically distinct from naturally occurring *Op*MNPV. To separate the mortality caused by the applied virus from that caused by the naturally occurring virus, a rare and genetically distinct strain of *Op*MNPV must be applied. To learn more about the genotypic diversity of *Op*MNPV populations in BC and to identify rare strains in this region, viral DNA was extracted from larvae reared from 208 field-collected egg masses found in five geographic regions of British Columbia and subjected to REN analysis. Nine, 12, and 9 different genotypes were detected using *Pst*I, *Sal*I, and *Hin*dIII, respectively. When the *Pst*I, *Sal*I, and *Hin*dIII profiles for each pure (single strain) isolate were grouped and considered as a combined *Pst*I–*Sal*I–*Hin*dIII genotype, 23 different genotypes were identified among 185 isolates. Nine rare *Op*MNPV genotypes were selected as ideal candidates for use as a potential 'marker strain' to accurately determine the efficacy of the treatment.


*Keywords:* *Orgyia pseudotsugata*; Nucleopolyhedrovirus; *Op*MNPV; *Pseudotsuga menziesii*; Biological insecticide; Genotypic variation; Restriction endonuclease analysis; *Pst*I; *Sal*I; *Hin*dIII

## 1. Introduction

The Douglas-fir tussock moth, *Orgyia pseudotsugata* McDunnough (Lepidoptera: Lymantriidae), is a native defoliator that feeds on the needles of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissener) Franco (Pinaceae)) and several true firs (*Abies* spp.) in the forests of western North America (Beckwith, 1978; Wellner, 1978). Outbreaks of *O. pseudotsugata* result in growth loss, top kill, and tree mortality (Alfaro et al., 1987; Wickman, 1978). Defoliated trees that survive the outbreak are weakened and are more susceptible to attack by other insects, particularly the Douglas-fir beetle, *Dendroctonous pseudotsugae* Hopkins (Coleoptera: Scolytidae) (Harris et al., 1985).

*Orgyia pseudotsugata* populations are cyclic, with an increase in population every 7–11 years in western North America (Otvos and Shepherd, 1991; Shepherd et al., 1984). Each outbreak generally lasts 2–4 years (Mason and Luck, 1978) and ends with a sudden collapse due primarily to an epizootic caused by a nucleopolyhedrovirus (NPV) (Baculoviridae) that occurs naturally within *O. pseudotsugata* populations. However, outbreaks usually collapse after extensive damage has already occurred in the infested stand (Dahlsten and Thomas, 1969; Shepherd and Otvos, 1986).

Two nucleopolyhedroviruses were found to infect Douglas-fir tussock moth populations in different areas

* Corresponding author. Fax: +1 250 363 0775.
*E-mail address:* iotvos@pfc.forestry.ca (I.S. Otvos).

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of the host range. A single embedded NPV (*Op*SNPV) occurs throughout the host range, while a multiple-embedded NPV (*Op*MNPV) is found in British Columbia (BC), Washington, Idaho, Oregon, and Montana (Hughes, 1976; Hughes and Addison, 1970). These two viruses have been demonstrated to have 1% or less DNA sequence homology (Rohrmann et al., 1978).

To control Douglas-fir tussock moth infestations, *Op*MNPV was developed by the USDA Forest Service for use as a viral insecticide (Martignoni, 1999). This insecticide was registered under the trade name TM Biocontrol-1 in the US in 1976, and in Canada in 1983. The same virus was also produced in the whitemarked tussock moth, *Orgyia leucostigma* (Smith) and registered under the trade name Virtuss in Canada in 1983 (Otvos et al., 1998). The last Douglas-fir tussock moth outbreak in British Columbia, from 1990 to 1993, was controlled by aerial application of TM Biocontrol-1 to the infested stands (Otvos et al., 1998).

Following application of TM Biocontrol-1 or Virtuss, mortality levels of Douglas-fir tussock moth larvae are monitored, but currently it is not possible to distinguish between mortality caused by the applied viral insecticide and mortality caused by naturally occurring strains of *Op*MNPV. To quantify the proportion of insect mortality due to the applied virus, it would be necessary to apply either a genetically altered virus or a unique strain in an area where that strain is not known to occur. The latter approach is preferable, as it would release a naturally occurring variant rather than a genetically altered virus.

Little is known about the source and maintenance of genotypic variation in baculoviruses. Several recent studies have definitively shown that homologous recombination between variants during baculovirus replication is a highly frequent event (Hajos et al., 2000; Kamita et al., 2003; Martin and Weber, 1997), and two or more heterogeneous strains of baculovirus infecting a single host larva are commonly found. Other possible sources of variation include acquisition of host cell DNA (Fraser et al., 1983) and mutations resulting in sequence deletion and/or reiteration within the viral genome. Genotypic changes appear to be significantly higher in regions of baculovirus genomes in which reiterated sequences have been shown to occur (Crawford et al., 1986).

A thorough assessment of the genotypic variation of baculovirus populations over a relatively localized geographic area could provide useful information relating to baculovirus evolution, but very few such studies have been conducted on this. Cherry and Summers (1985) compared REN patterns of 21 wild isolates of *Spodoptera littoralis* (Boisduval) NPVs in Israel and found two equally distributed distinct virus groups. Their study did not assess whether genotypic variation and geographic location were correlated within the two subgroups. Shapiro et al. (1991) compared 22 wild isolates of *Spodoptera frugiperda* (Smith) NPV from 15 isolates in Louisiana and seven other locations around the world, and found that genetic variation among foreign isolates was greater than among Louisiana isolates. Within agricultural fields, there was significant variation in *Sf*NPV, but this variation was not correlated to the insect's host plant species. The study was not designed to test whether there was a correlation between the degree of genetic differentiation and geographic distance within Louisiana. Cooper et al. (2003) examined the genetic diversity of *Malacosoma californicum pluviale* (Dyar) (western tent caterpillar) NPVs at different spatial scales, and found that, at least at low host densities, virus variants from within families (tents) were more likely to be the same than isolates among populations (sites), and isolates within populations were more likely to be the same than isolates collected on neighbouring Gulf islands near Victoria, BC. They noted the potential for viruses to become locally adapted to hosts in different sites, although no dominant genotype was observed at any site (Cooper et al., 2003).

Laitinen et al. (1996a) initiated a survey of *Op*MNPV genotypic variation in the southern interior of British Columbia, Canada. Ninety viral DNA samples were extracted from dead larvae from 10 of the original 42 sites where egg masses were collected in this region. Restriction digests with the enzymes *Pst*I and *Sal*I showed five different *Pst*I genotypes and two different *Sal*I genotypes among these viral samples.

The purposes of our current study were to (1) complete the survey of *Op*MNPV genotypic variation in British Columbia and use this molecular information to compare virus isolates from different sites and regions to discern geographical patterns or population substructure, and (2) to identify and select naturally occurring, rare and genetically distinct variants as candidates for use in an experiment to determine the proportion of mortality caused by the application of these unique strains in an area where they do not naturally occur. The efficacy of the candidate strains are being determined in a separate study in the laboratory. *O. pseudotsugata* mortality caused by aerial application of one unique virus strain in a tussock moth-infested stand where it does not occur will then be partitioned from mortality caused by naturally occurring virus at the test site by determining the proportion of dead larvae containing the distinct genotype of the applied *Op*MNPV strain.

## 2. Materials and methods

### 2.1. Collection and rearing of Douglas-fir tussock moth larvae naturally infected by OpMNPV

Douglas-fir tussock moth egg masses were collected from 42 sites in six geographical regions in British