

BIOLOGICAL INSECTICIDES FOR MANAGING DOUGLAS-FIR TUSSOCK MOTH IN CONIFER HOST FORESTS

-- *QUESTIONS AND ANSWERS* -

What is the Douglas-fir tussock moth virus?

Wild populations of the Douglas-fir tussock moth have several naturally-occurring diseases that cause mortality to certain life stages of the insect during the course of an outbreak. The agents that cause these diseases are bacteria, fungi, and viruses, among others. Research during the 1960's to mid-1980's, mostly by U. S. Forest Service scientists, determined that a nucleopolyhedrosis virus, or NPV for short, (family Baculoviridae; genus *Baculovirus* Subgroup A), caused considerable mortality of tussock moth larvae during the later stages of tussock moth outbreaks (3rd and 4th year of an outbreak), and was largely responsible for natural collapse of many of the tussock moth outbreaks that occurred in western North America.

Scientists at the Pacific Northwest Research Station at Corvallis, Oregon began investigations with the tussock moth NPV during an accelerated research program to bring on line, new technologies to manage tussock moth populations after a Douglas-fir tussock moth outbreak that was occurring in large areas of eastern Washington, northeastern Oregon, and parts of Idaho during the period 1971-1974 was treated with DDT under special approval by the U. S. Environmental Protection Agency. Laboratory and field research on virus efficacy against tussock moth, and specific safety testing in various organisms that showed no adverse effects, eventually led to the registration of the tussock moth NPV by the U. S. Environmental Protection Agency in 1976 as a biological insecticide under the name TM BioControl-1. The Forest Service produced the virus and stockpiled large quantities (over 400,000 acre doses) during the period 1985-early 1990s, in anticipation of possible use the next time tussock moth was in outbreak.

To date, the U. S. Forest Service has used only a small amount of TM BioControl-1 to operationally treat a tussock moth outbreak in Idaho. Most of the uses of the virus in North America have been by the Canadian Forest Service against tussock moth outbreaks that have occurred in British Columbia, Canada.

The tussock moth NPV is a DNA virus in that it uses the DNA contained within nuclei of certain insect host cells in order to replicate itself. The virus particles themselves (or virions) are bundled together and embedded in a crystalline protein material to form polyhedral inclusion bodies which protect the virus particles until they are ingested by larvae of Douglas-fir tussock moth.

How is the Douglas-fir tussock moth virus transmitted?

Douglas-fir tussock moth larvae have to ingest the virus in order for it to infect the larval host. The virus requires a living host or the DNA of live host cells in order to replicate itself. Under field conditions, tussock moth larvae ingest the polyhedral inclusion bodies containing virions or virus particles along with their food—the needles of host trees (including Douglas-fir, grand or white fir, and certain other true fir species) when the host foliage is inoculated with the virus. After ingestion, the inclusion body protein is solubilized in the alkaline condition of the gut of the insect, and soon the virions are released from the crystalline protein matrix and penetrate the cells lining the midgut. These midgut cell nuclei are where the first cycle of replication begins in the insect's body. A number of days after eating foliage containing the virus, infected larvae become sluggish and eventually cease feeding shortly before they die, usually between 7-14 days. By the time larvae die, viral replication is complete and larvae become flaccid as tissues begin breaking down. The numbers of polyhedral inclusion bodies have now increased exponentially through replication in the insect's cell nuclei. The chitinous integument of the larva becomes so fragile due to breakdown by autolytic enzymes that it is easily ruptured by movement of the branch containing the now dead, virus-infected larval cadavers. When this occurs, the virus laden contents within the insect are spilled out and contaminate the host foliage below. This contaminated foliage then becomes the route for further transmission of virus in the population, as other tussock moth larvae consume the foliage and ingest the virus polyhedral inclusion bodies adhering to the foliage. Hence, contagion is the process by which the virus spreads through the tussock moth population. Rain and spring snow melts help to distribute the virus over the foliage so that the next generation of tussock moth larvae have an increased likelihood of encountering the virus-contaminated foliage.

What makes this virus safe to use?

The nucleopolyhedrosis virus of the Douglas-fir tussock moth is quite literally "a part of nature." It is and has always been an important component of the forest environment. It is naturally occurring, and is not something new that is being introduced into the ecosystem.

Nucleopolyhedrosis viruses only occur in arthropods, and individual NPVs are very limited in their host range. The Douglas-fir tussock moth NPV, for example, is only effective against Douglas-fir tussock moth and 2 or 3 other related tussock moth species. The tussock moth NPV does not infect any other insect species, including beneficial insects, nor does it infect animals including fishes and birds.

NPVs have been certified for use as insecticides in North America by the U. S. Environmental Protection Agency (EPA), and the Environmental Protection Branch (EPB) of Environment Canada. Used as microbial insecticides, NPVs do not interact with non-target organisms in forest, agricultural, or aquatic ecosystems. NPVs are nonpolluting and biodegradable in the environment. The small amounts applied in aqueous formulations to forest canopies (less than one-half to three grams per acre) eventually become inactivated by exposure to sunlight (UV

radiation), or are washed from the foliage into the duff layer or the mineral soil by rain and melting snow. There it is slowly denatured by low soil pH.

What tests have been conducted to show that NPVs are not harmful to fishes?

The Douglas-fir tussock moth nucleopolyhedrosis virus, as part of the safety testing required for registration by the EPA, was tested on three species of salmonid fishes to determine any adverse effects. Chinook salmon, coho salmon, and steelhead trout showed no pathology when exposed to *Baculovirus* (Douglas-fir tussock moth NPV) by three different routes (waterborne, feeding, and injection). Cell lines derived from chinook salmon and steelhead trout were refractory to nonoccluded virions, and no cytopathology was observed by light and electron microscopy. Both polyhedra and nonoccluded virions were inactivated rapidly by coho salmon exposed to the virus by the three different routes. The virus survived less than 24 hours in coho salmon when exposed to the agent by the waterborne route, feeding, or injection. It also appeared that coho salmon might not eat tussock moth larvae when other food was available.

In addition to these studies, there have been other tests to determine the effects of insect viruses on fishes and amphibian inhabitants of aquatic or semi-aquatic ecosystems. Test organisms have included a dozen fish species including, trout, bullheads, suckers, killifish, spot, sheepshead, and others. None of these organisms have shown any adverse effects when exposed to insect viruses. In other tests involving fish and amphibian tissue culture, there was no evidence that NPV replicated in any cell from a cold-blooded vertebrate, including cells from adult and embryonic rainbow trout, juvenile brown bullhead, the bluegill, adult fathead minnow, and adult bullfrog; all of which eat insects. Studies of 240 juvenile bluegills and 240 brown trout exposed for 96-h to gypsy moth NPV at concentrations 100 times higher than those normally effective against that insect established a total absence of harmful effects. These negative results are consistent with the biology of animal viruses. There are no known vertebrate counterparts to nucleopolyhedrosis viruses of insects. These results all demonstrate that viruses of the Baculoviridae are nontoxic to fish and other aquatic amphibians.

What tests have been conducted to show that NPVs are not harmful to nontarget invertebrates in aquatic environments?

Insect virus candidates have been tested in exposure trials using marine invertebrates like oysters and shrimp with no adverse effects under the test conditions used. Tests have included 96-h acute exposures, 10-day maximum exposures, and 7-day exposures at various dosages. Both brown shrimp and oysters have been tested in both feeding and inoculation studies using NPV. All tests showed no adverse effects. Electron microscopy found no evidence of virus particles in the cells of either the oysters or the shrimp. Crayfish, grass shrimp, mollusks, *Daphnia magna*, *Chironomus thummi*, *Notonecta undulata*, waterboatmen, and water fleas have also been exposed to NPV with no reported adverse effects.

What tests have been conducted to show that NPVs are not harmful to nontarget vertebrates?

Insect virus candidates have been tested in exposure trials using various animals since the 1960s. The NPV of an agricultural insect, the corn earworm (*Heliothis zea*), was one of the first insect viruses to undergo testing for registration with EPA. The safety tests were as extensive as any required for chemicals by the EPA at the time, and included long-term carcinogenicity and teratogenicity studies on man and other primates. These tests proved negative. Over the years, NPVs have been tested and shown unable to replicate or cause pathogenic effects on microorganisms, noninsect invertebrate cell lines, vertebrate cell lines, vertebrates, plants, and nonarthropod invertebrates. The animal organisms tested included (besides humans and other primates) mouse, rat, guinea pig, rabbit, dog, quail, chicken, house sparrow, mallard duck, pheasant, deer, immunosuppressed rats, and several species of fish (see above). Most tests were conducted at doses 10 to 100 times the average field application rate per acre converted to a ratio of weight of test animal to weight of a 70-kg human. Exposure routes included oral, inhalation, dermal application, intradermal, intramuscular, intra cerebral, intravenous, intra peritoneal, and subcutaneous. A recombinant *Baculovirus* also has been tested and found to be unable to enter and express viral DNA in mammalian cells that are considered refractory to baculoviral replication. This test demonstrated that insect Baculoviruses have an inherent level of safety with regard to mammalian species.

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