RESPONSE OF THIRD-, FOURTH-, FIFTH-, AND SIXTH-INSTAR SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.), LARVAE TO NUCLEAR POLYHEDROSIS VIRUS

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Four different types of insect viruses have been isolated from the eastern spruce budworm, *Choristoneura fumiferana* (Clem.), but most research efforts have been concentrated on developing the nuclear polyhedrosis virus (NPV) as a viable control agent (Cunningham 1985). There are no published reports of LD_{50} values for this important forest pest. Recently, a LC_{50} of 483 viral polyhedra per square millimetre of diet surface for fifth-instar budworm larvae was determined using surface contamination feeding techniques (Cunningham *et al.* 1983). Because the dosages used in efficacy trials are derived from laboratory LD_{50} values, experiments were conducted to determine the virulence of this NPV to eastern spruce budworm. Bioassays were conducted with all feeding instars. Reported here are values for the LD_{50} dosages for third-, fourth-, fifth-, and sixth-instar spruce budworm larvae.

Spruce budworm larvae were obtained from the insect rearing facilities of the Forest Pest Management Institute, Sault Ste. Marie, Ont. Virus was purified from spruce budworm larvae infected with the "wild-type" isolate of NPV found naturally in eastern spruce budworm populations and routinely propagated in this laboratory. This was confirmed by restriction endonuclease digestions using the Hind III enzyme (Arif and Brown 1975; Arif and Doerfler 1984). Stock suspensions of NPV were standardized using a dry counting method (Wigley 1980). After inspection of preliminary infection data, five serial dilutions of NPV were made from the stock suspension ranging below and beyond the estimated lethal dosage for each larval instar. For bioassay, 2 μL of each dilution was placed onto a small pellet of artificial diet (McMorran 1965) inside a Beem embedding capsule. Immediately afterward, a newly molted budworm larva of appropriate instar was placed into the capsule to feed on the contaminated diet. After 48 h, larvae that had consumed the entire pellet were transferred to individual cups of diet and placed in rearing chambers (22°C; 60% RH) until death or adult emergence. Daily observation scored deaths. Only those larvae that had died from NPV infection, as determined by microscopic examination, were included in the analysis. Dilutions, including an untreated control, were replicated between three and 15 times depending on the number of spruce budworm larvae available. Twenty-five larvae were used in each replicate (75–375 insects per dilution). Results were analyzed using SAS Probit to calculate the LD_{50} and 95% fiducial limits (SAS Institute 1985).

LD_{50} dosages of NPV for third-, fourth-, fifth-, and sixth-instar budworm larvae were calculated to be 455, 1141, 13 016, and 36 554 viral polyhedra, respectively (Table 1). LD_{50} values were not determined for the non-feeding first-instar budworm larvae and the second-instar budworm larvae which seldom consumed an entire diet pellet before molting. Spruce budworm overwinters as a second-instar larva and in spring molts to a third-instar larva to mine either needles or buds. Feeding on the buds continues in the fourth-, fifth-, and sixth-instar larvae which are the primary targets for control to protect foliage (Morris 1963).

No direct comparison can be made between the LD_{50} of 1141 viral polyhedra and LC_{50} dosage for fourth-instar larvae because of the differences in the methodologies used to determine the dose–response and the lack of information on the feeding habits of spruce budworm. Although identical diets were used in both tests, results by Cunningham *et al.* (1983) do not take into account any variation in the distribution of viral polyhedra on the diet surface, different feeding rates of the larvae, and any interactions between larvae

Table 1. LD_{50} dosage of nuclear polyhedrosis virus to eastern spruce budworm, *Choristoneura fumiferana* (Clem.)

Instar	LD_{50}*	95% fiducial limits	
		Lower	Upper
III	455	144	605
IV	1 141	643	1 883
V	13 016	9 585	18 684
VI	36 554	27 869	50 391

*Expressed as viral polyhedra.

reared in the same container. The method of dosing described here reflects most closely the way in which the insect would encounter virus in nature. Retnakaran (1983) observed that approximately 2 mg of this diet is consumed by a fourth-instar larva. If this quantity approaches the 2 mm^2 surface area consumed, then the values for the LD_{50} and LC_{50} are similar.

Specific dose–response data are required to assess the potential of NPVs as biocontrol agents. They are also required to determine the correct application rate for field efficacy testing. Spruce budworm NPV has been applied at rates from 2.5×10^{10} to 5.7×10^{12} viral polyhedra per hectare; these dosages have been determined empirically because complete LD_{50} data were not available (Cunningham and Howse 1984; Kaupp *et al.* 1990). This may be of significance in explaining the unacceptable results found using this NPV as a biological insecticide at current recommended dosage rates (Kaupp *et al.* 1990). Perhaps better efficacy would be achieved if a greater amount of virus was applied in view of these LD_{50} values. This would require additional research into technology to apply large dosages effectively, as well as better facilities to produce this NPV more efficiently.

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