PATHOGENICITY OF THE IRELAND STRAIN OF NUCLEAR POLYHEDROSIS VIRUS TO SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA*, LARVAE

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The spruce budworm, *Choristoneura fumiferana* (Clem.), nuclear polyhedrosis virus (*Cf*MNPV) has been studied extensively for its potential use as a bio-insecticide (Cunningham 1995). Recent advances in recombinant DNA technology have been impetuous in the genetic engineering of this virus to increase its virulence and (or) speed of action. This aspect of research has been concentrated on the modification of a plaque purified (Ireland strain) isolate obtained from the wild-type virus population (Arif et al. 1984). To assess any improvement in the effectiveness against the target pest, the virulence of the unaltered virus must first be determined. Bioassay results that have been previously reported by Kaupp and Ebling (1990) were conducted on the wild-type virus found in nature which consists of a mixture of viruses (Arif et al. 1994). Results of bioassays conducted to determine dose–response and time–response of fifth-instar spruce budworm larvae to the Ireland strain of *Cf*MNPV are reported here.

The Ireland strain of *Cf*MNPV was passaged through laboratory-reared larvae to produce fresh inoculum for testing. Occlusion bodies (OBs) were collected from cadavers by filtration and centrifugation (Arif and Brown 1975), enumerated using a stained film method (Wigley 1980), and stored at 4°C until bioassay. Dose–response and time–response of fifth-instar spruce budworm larvae to the nuclear polyhedrosis virus (NPV) was determined using an inoculated diet plug bioassay technique similar to that of Kaupp and Ebling (1990).

Dose–response was determined by the feeding of inoculated diet plugs by fifth-instar spruce budworm larvae. NPV inoculations targeted 5, 80, 85, 90, and 95% mortality, giving the narrowest fiducial limits for both the LD_{50} and LD_{95} (Robertson and Preisler 1992), estimates of the dosages required to kill 50 and 95% of the test larvae, respectively. Based on preliminary bioassays, dosages of 2.570×10^2, 9.692×10^4, 1.543×10^5, 2.768×10^5, and 6.586×10^5 OBs in a 2-μL volume were placed onto a 4-mg pellet of artificial diet (McMorran 1965) inside a Beem® embedding capsule. Immediately thereafter, a newly molted (less than 24 h) fifth-instar larva, reared on a formaldehyde-free diet at 25°C, 60% RH, and 16L:8D light–dark photoperiod, was placed into the capsule to feed on the treated diet. After 24 h, larvae that had consumed the entire pellet were transferred to individual cups of diet and placed in rearing chambers (25°C, 60% RH, 16L:8D light–dark photoperiod) until death or pupation. Larvae that did not consume the entire pellet were excluded from the experiment. Daily observation scored death, and only those larvae which died from NPV infection, as determined by microscopic examination, were included in the analysis. Larvae were considered to be dead when showing no movement after being gently prodded with a probe. It was assumed that larvae attaining the pupal stage showed no response to the inoculum. Bioassay of the five dilutions and an untreated control were replicated three times using 50 larvae for each replicate. Diet was changed weekly. Results were analyzed using probit analysis (LeOra Software 1994) to estimate LD_{50}, LD_{95}, and their associated fiducial limits.

Time–response was also determined by the feeding of inoculated diet plugs by fifth-instar spruce budworm larvae. Using an independent sampling design, five separate groups of test subjects were treated using the LD_{95} dose determined by the dose–response bioassay. Each group was then observed for a different period of time, and numbers of responses recorded at each observation period. The observation periods targeted 5, 80, 85, 90, and 95% mortality. Targeting these times (observation periods) gave the narrowest fiducial limits for both the ST_{50} and ST_{95} (Robertson and Preisler 1992), estimates of survival times of 50 and 95% of the test larvae, respectively. The observation periods, estimated using the dose–response bioassay, were 5, 9, 10, 11, and 12 days. For bioassay, a LD_{95} dose (1.802×10^5 OBs) in a volume of 2 μL was used, following the same bioassay method and rearing conditions as those described above for dose–response. Only those larvae which died from NPV infection, as determined by microscopic examination, were included in the analysis. Responses that occurred in a given group

Table 1. Dose–response and time–response of fifth-instar Choristoneura fumiferana to the Ireland strain of nuclear polyhedrosis virus

	Level of response		
LD_{50}	LD_{95}	ST_{50}	ST_{95}
4.340×10^3	1.802×10^5	7.8	12.9
$(2.329 \text{ to } 7.355 \times 10^3)$	$(1.052 \text{ to } 3.425 \times 10^5)$	$(7.1–8.4)$	$(11.9–14.6)$

NOTE: Level of response was calculated using probit analysis (LeOra Software 1994). Data for ST_{50} and ST_{95} were obtained using a LD_{95} dose of 1.802×10^5 OBs. Values in parentheses are 95% fiducial limits.

were recorded only once. Time–response data were analyzed using probit analysis (LeOra Software 1994), with time replacing dose, to estimate the 50 and 95% survival times of the test larvae (ST_{50} and ST_{95}) and the associated fiducial limits

Because variation in size of larvae among populations can affect bioassay results (Ebling and Kaupp 1997), the live body mass of 100 fifth-instar spruce budworm larvae, from the same population of larvae used for bioassay, was determined. Mean live body mass ($\pm$SD) was determined to be 7.3 ± 2.3 and 6.9 ± 2.4 mg for the dose–response and time–response bioassays, respectively. Future comparison of other strains of NPV to the Ireland strain should be standardized by using spruce budworm larvae with similar body mass.

The LD_{50} and LD_{95} of the Ireland strain to fifth-instar spruce budworm were estimated to be 4.340×10^3 and 1.802×10^5 OBs, respectively (Table 1). The slope of the regression line was 1.016 ± 0.061. The ST_{50} and ST_{95} of the Ireland strain were estimated to be 7.8 and 12.9 days, respectively (Table 1), when using a LD_{95} dose. The slope of the regression line was 7.561 ± 0.551.

Kaupp and Ebling (1990) estimated the LD_{50} of the wild-type mixture of NPV to fifth-instar spruce budworm larvae to be 1.302×10^4 OBs. The relative potency of the wild-type mixture to the Ireland strain is estimated (LeOra Software 1994) to be 0.336. This difference can be explained by the fact that the wild-type mixture consists of several strains, including the Ireland strain, each having different virulence. The Ireland strain has greater virulence (lower LD_{50}) than the combined mixture of strains previously bioassayed.

Results presented here form the basis upon which any genetically modified *Cf*MNPV should be compared.

Arif, B.M., and K.W. Brown. 1975. Purification and properties of a nuclear polyhedrosis virus from *Choristoneura fumiferana*. *The Canadian Journal of Microbiology* 21: 1224–1231.

Arif, B.M., P. Jamieson, S.S. Sohi, W. Kaupp, and J.A. MacDonald. 1994. Symbiotic-like relationship between two MNPV's of the spruce budworm, *Choristoneura fumiferana*. pp. 145–146 *in* Abstracts, Vol. II, VIth International Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, France. Society for Invertebrate Pathology.

Arif, B.M., J. Kuzio, P. Faulkner, and W. Doerfler. 1984. The genome of *Choristoneura fumiferana* nuclear polyhedrosis virus: molecular cloning and mapping of the EcoRI, BamHI, SmaI, XbaI and BglII restriction sites. *Virus Research* 1: 605–614.

Cunningham, J.C. 1995. Baculoviruses as microbial insecticides. pp. 261–292 *in* Reuveni, R. (Ed.), Novel Approaches to Integrated Pest Management. CRC Press, Boca Raton, Florida.

Ebling, P.M., and W.J. Kaupp. 1997. Pathogenicity of a nuclear polyhedrosis virus to forest tent caterpillar, *Malacosoma disstria* (Hübner) (Lepidoptera: Lasiocampidae). *The Canadian Entomologist* 129: 195–196.

Kaupp, W.J., and P.M. Ebling. 1990. Response of third-, fourth-, fifth-, and sixth-instar spruce budworm, *Choristoneura fumiferana* (Clem.), larvae to nuclear polyhedrosis virus. *The Canadian Entomologist* 122: 1037–1038.

LeOra Software. 1994. POLO-PC: a user's guide to Probit Or LOgit analysis. LeOra Software, 1119 Shattuck Ave., Berkeley, California.

McMorran, A. 1965. A synthetic diet of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). *The Canadian Entomologist* 97: 58–82.

Robertson, J.L., and H.K. Preisler. 1992. Pesticide bioassays with arthropods. CRC Press, London.

Wigley, P.J. 1980. Counting micro-organisms. pp. 29–35 *in* Kalmakoff, J., and J.F. Longworth (Eds.), Microbial Control of Insect Pests. *New Zealand Department of Scientific and Industrial Research Bulletin* 228.