# BIOASSAY OF Choristoneura fumiferana NUCLEAR POLYHEDROSIS VIRUS PRODUCED FROM THE CF-124T CELL LINE

File Report No. 95

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### INTRODUCTION

Recent investigations into the infectivity of Eastern spruce budworm, Choristoneura fumiferana Clem. nuclear polyhedrosis virus (NPV) produced in tissue culture have shown astonishing differences in the infectivity of this virus as compared to naturally occurring wild type NPV. Virus purified from the CF-70 cell line was very significantly reduced in infectivity. This reduction in infectivity was observed to occur after only one passage.

Dr. P. Faulkner, of Queens' University has also been working with spruce budworm NPV but this virus is produced in a cell line originally obtained from our laboratory, namely IPRI-CF-124T cells. Because of the changes experienced with the CF-70 cell line produced NPV, virus purified from the CF-124T cell line was bioassayed to document any in-vitro derived reduction in infectivity.

# **METHODS**

Serial dilutions were prepared from purified NPV obtained from infected CF-124T cells. Concentrations ranged from  $1.04 \times 10^5$  polyhedral inclusion bodies (PIBs)/mL to  $1.04 \times 10^7$  PIBs/mL.

For bioassay, 2uL of each dilution was placed onto a small pellet of artificial diet inside a Beem embedding capsule. Immediately afterwards a fourth instar eastern spruce budworm, *C. fumiferana* larva was placed inside the capsule to feed on the contaminated diet. After 48 h larvae that had consumed the entire pellet of diet were transferred individually to a cup of diet, and placed in rearing chambers at 70°C, 60% R.H. until death or adult emergence. Any non-virus deaths observed

within four days of being transferred onto diet were attributed to handling. Daily observation scored deaths. All dead larvae were examined microscopically for NPV infection, and only those that died from virus were included in determination of LD $_{50}$ . Data from six dilutions, including an untreated control, replicated three times were used to determine the LD $_{50}$  for this NPV isolate.

### RESULTS AND DISCUSSION

Results from the bioassay determined that the LD $_{50}$  for virus derived from CF-124T cells was 4803, significantly higher than the LD $_{50}$  of 1140 for the wild type NPV (Table 1). However, general observations indicated that the virulence of this isolate was similar to that of the wild type.

It is interesting to note the general reduction in infectivity of NPV produced in tissue culture. More research is needed to determine whether this phenomena is widespread in cell systems. If so, such reduction in infectivity caused by in-vitro production may be detrimental to the concepts of mass production of insect viruses in cell culture systems.

Table 1. Lethal dosage of nuclear polyhedrosis virus isolates to fourth instar Choristoneura fumiferana larva.

	95% fiducial			
Isolate	LD <sub>50</sub>	limits		
CF-124T	4803	3346-6648		
Wild Type	1140	642-1883		

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