BIOASSAY OF A COMMERCIALLY PREPARED PREPARATION OF TM-BIOCONTROL 1

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INTRODUCTION

TM-BioControl 1 is a nuclear polyhedrosis virus (NPV) product which infects the Douglas-fir Tussock moth, *Orgyia pseudotsugata* (McDunnough). It was originally produced, and registered in the U.S., by the USDA Forest Service. Large numbers of NPV-infected larvae have been stored frozen in preparation for future outbreaks of this forest defoliator.

Recently, Espro Inc. purchased these stocks of frozen larvae and has attempted to prepare a semi-purified product for commercial use. A sample from a test batch of this commercial preparation was bioassayed to determine if any deleterious effect on the infectivity of the virus occurred during purification.

METHODS

Serial dilutions were prepared from virus suspensions of the semi-purified material and from unprocessed, frozen larvae. Concentrations ranged from 1.25×10^4 polyhedral inclusion bodies (PIBs)/mL to 3.31×10^5 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 second instar white-marked tussock moth (*O. leucostigma* (J.E. Smith)) 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 four dilutions, including an untreated control, replicated three times were used to determine the LD_{50} for each sample. The entire bioassay was repeated to ensure reliability of the results.

RESULTS AND DISCUSSION

There was no difference between the LD_{50} dosages determined from each repeat of the bioassay (Fig. 1). This indicates that the insects were of homogeneous nature and any observable differences can be attributed to the purification procedure. Results indicate that the commercial processing does have a significant effect on the infectivity of the virus as compared to the infectivity of the NPV in the unprocessed material. The LD_{50} dose using the semi-purified material was found to be 202 PIBs while the LD_{50} dose using the unpurified product was 85 PIBs (Table 1). This represents a considerable decrease in the infectivity of the PIBs due to the treatment process.

On examination of the quantities of virus required to cause higher mortality, we find the same effect, with the LD_{95} dose for the commercial preparation at 3104 PIBs and for the frozen material at 1114 PIBs. Although these values may not be statistically different, they indicate a deleterious effect of the processing on the NPV of *O. pseudotsugata*.

Table 1. Lethal dosages of *O. pseudotsugata* NPV from processed and unprocessed material bioassayed with second instar *O. leucostigma* larva.*

	Unprocessed material	95% fiducial limits	Processed material	95% fiducial limits
LD_{50}	85	55 - 122	202	121 - 313
LD_{95}	1114	630 - 2866	3104	1424 - 15556

* Analysis of results from two tests.

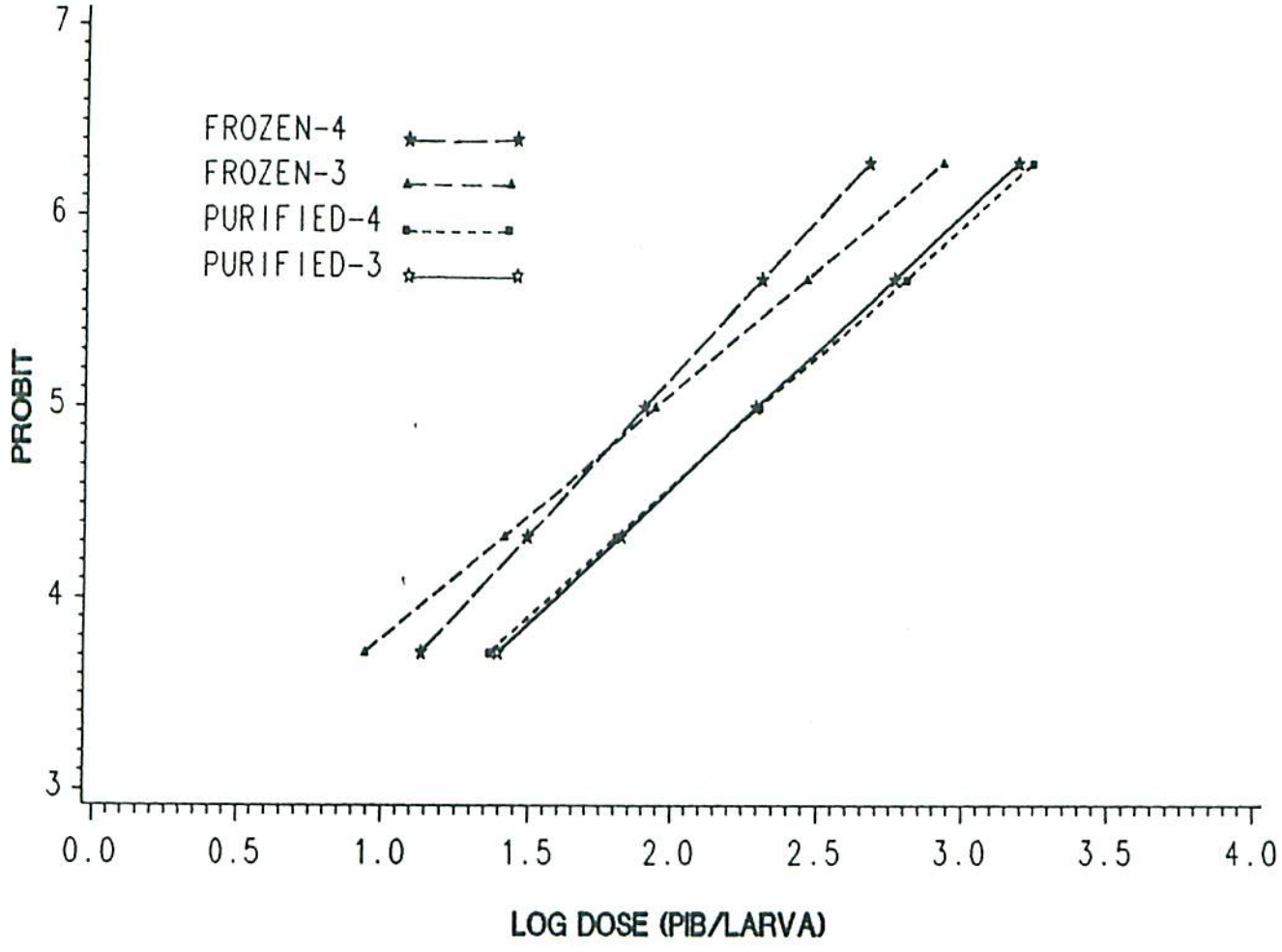

Fig. 1. Response of second instar *Orgyia leucostigma* larvae to processed and unprocessed *O. pseudotsugata* NPV illustrating the homogeneous nature of the insects.