SAFETY TESTING OF A NUCLEAR POLYHEDROSIS VIRUS FOR USE AGAINST GYPSY MOTH, *LYMANTRIA DISPAR*, IN NEW ZEALAND

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ABSTRACT

Gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), is a serious pest of forests and horticulture in the northern hemisphere. Viable egg masses have been found on ships in New Zealand ports. If gypsy moth does reach onshore, it is important that measures for its eradication have minimal negative environmental impact. A microbial pathogen used to control gypsy moth with success is a nuclear polyhedrosis virus (LdNPV). This virus appears to be relatively specific, attacking only *L. dispar* in the field. It was imported to New Zealand to determine its safety against Lepidoptera, especially endemic species. Twenty species of endemic and introduced Lepidoptera were exposed to LdNPV, without visual evidence of infection. This was confirmed by probing any dead treated caterpillars using labelled LdNPV DNA to detect latent infections.

Keywords: gypsy moth, *Lymantria dispar*, nuclear polyhedrosis virus, environmental safety, host range

INTRODUCTION

Gypsy moth (GM) is a major lepidopteran pest of native and plantation forests and horticultural tree crops throughout the northern hemisphere. GM was accidentally released around Boston, USA, in 1869 and has since spread throughout the northeast and into Canada (Montgomery and Wallner 1988). Females of this strain of GM are flightless, which has limited its spread. Recently, an "Asian" strain of gypsy moth (AGM), thought to have originated in Siberia, has been spreading throughout northern Asia and Europe. This strain has females which fly before egg laying, as well as having other characteristics (such as increased host range) which increase the potential pest status. The finding of viable AGM egg masses on ships in New Zealand ports in the last few years has raised awareness of the danger of this pest reaching our shores. Risk assessments by the Ministries of Forestry (MoF) and Agriculture (MAF) and the Forest Research Institute (FRI) concluded that it is almost inevitable that gypsy moth will reach New Zealand and that it would be a devastating pest (Cowley *et al.* 1993; Horgan 1994).

Biological control is a well established method of GM control in the USA, utilising several pathogens. The lepidopteran-active bacterium, *Bacillus thuringiensis* var. *kurstaki*, is used in several biological insecticides and is lethal to the GM larval stage (Reardon *et al.* 1994). It is also registered as a control agent in New Zealand, mainly for use against leafrollers. Although its specificity to Lepidoptera is preferable to a broad spectrum insecticide, the death of non-target larvae, especially rare species, makes its use in ecologically-sensitive areas unacceptable. Resistance to *B. thuringiensis* (Bt) has also been reported for some insects (eg. McGaughey 1985). Therefore additional, specific control agents would greatly assist in eradication or control of GM in this country.

A pathogen which has been successful in controlling pest outbreaks in the northern hemisphere forests is the gypsy moth nuclear polyhedrosis virus (LdNPV). The virus is a member of the genus *Baculovirus*, and was first registered as a biological pesticide in 1978 in the USA (Reardon and Podgwaite 1992, 1994). Isolates of this virus have been produced for gypsy moth control in the USA, Canada, Czech and Slovak

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Republics and the "USSR". In China, a NPV routinely causes 50% natural mortality among GM populations (Yan *et al.* 1994). As the virus is produced commercially and applied to large areas of forest, it appears a good candidate for use in New Zealand. The main benefit of the virus over Bt and other control measures is its specificity. Overseas, extensive research has shown that the virus attacks only *L. dispar* and possibly another Lymantriidae species (Barber *et al.* 1993), indicating that any danger of use in New Zealand is likely to be restricted to Lepidoptera. New Zealand has no Lymantriidae species (Dugdale 1988), the closest families taxonomically are Noctuidae or Hepialidae. The objective of this study was to collect, rear, and dose species of New Zealand Lepidoptera with LdNPV to determine any susceptibility.

MATERIALS AND METHODS

Collection, maintenance and identification of larvae

Lepidoptera were collected directly from the field (by the authors or those listed in the acknowledgments), or obtained commercially (Table 1). All larvae were kept at 20°C under a 18:6 L:D photoperiod. Eggs collected from moths were placed in a container with damp filter paper and kept at 20°C for hatching. Larvae were used in all pathogen treatments. Where possible, larvae were reared through to adults for identification. Pupae were kept in containers at ambient temperature, with damp filter paper helping to maintain humidity until emergence. Larvae were identified according to Hudson (1928), Scott (1984) and Dugdale (1988). Where insects could not be identified, adults were sent to John Dugdale (Landcare, Auckland).

Inoculation of larvae with virus

Larvae were dosed in a 24 well Falconer tissue culture dish. A 5 mm bore of the leaf material was contaminated with 2 ml of NPV (1.5×10^5 polyhedral inclusion bodies [PIB]/2 ml) in 100 mM Phosphate Buffer Solution, pH 7.2 (PBS), or 2 ml of PBS only as a control. Food samples were left to absorb the droplets and then larvae were placed in the wells. Virus and untreated controls were alternated between rows of four wells. Larger larvae, such as *B. mori*, that were too big to fit in the wells were placed in individual specimen pottles (4.5 x 5 mm) containing the diet and treated in the same manner. Where the food was a granular diet (*G. mellonella*, *P. interpunctella*, *T. biselliella* and *H. pseudospretella*), 2 ml of virus or PBS was added to approximately 0.041 g of diet. Larvae were left at 20°C until they had consumed the food sample entirely. Generally, 7-10 days after inoculation, larvae from each treatment were transferred to communal containers, maintained at 20°C and monitored for the next 3-4 weeks. The proportions of larvae dying in the treated and control groups were compared using Chi-squared tests.

At the conclusion of the bioassays a sample of virus was sent to Dr John Podgwaite (US Forest Service, Hamden, CT, USA) for bioassay against *L. dispar* larvae (New Jersey laboratory strain F35), to confirm virulence of the virus. The sample from New Zealand was run at the same time as a standard virus strain, MR1-8. Two hundred and fifty newly moulted second instar larvae were treated with 10^2 to 10^6 PIB/ml incorporated in a high wheat germ diet (Bell *et al.* 1981) at 52°C, as described by Lewis and Rollinson (1978). Inoculated larvae were maintained at 24°C under a 16:8 L:D photoperiod, and checked daily for mortality. Lethal concentrations (LC) was calculated by probit analysis using POLO-PC program (LeOra Software, Berkeley, CA).

Probe detection of virus

All larvae which died after exposure to virus and some untreated larvae were examined for the presence of virus using ^{32}P -labelled LdNPV and a "slot blot" procedure. Insect cadavers were homogenised in a volume of PBS similar to that of the cadaver and 20-150 μl loaded onto pre wet (10 x SSC: 3M NaCl/0.3M tri-Sodium Citrate) nylon membrane (Hybond N, Amersham, NZ) using a Minifold II Slot-Blot apparatus (Scheicher and Schuell, Keene, N.H.) linked to vacuum. Twenty μl containing 10^0 , 10^3 , 10^4 , 10^5 , 10^6 and 10^7 PIBs of LdNPV were included as positive controls. PBS was loaded in at least 10 slots as negative controls in addition to untreated (control) cadaver macerates. After samples were loaded, the membranes were air dried, denatured with 0.5M NaOH/ 1.5M NaCl for 30 min. at 65°C, then neutralised for 5

min. at RT with 0.5M Tris pH 7.0/ 1.5M NaCl, treated for 5 min. in 10 x SSC and air dried for 30 min and baked for 20 min. at approximately 120°C. These membranes were stored for several days in a desiccator at 4°C. Membranes were subsequently prehybridised, hybridised and washed according to standard methods (Sambrook *et al.* 1989). DNA for labelling was isolated from purified virions of LdNPV using the method of Bourner (1993), from 500 µl of approximately 10⁸ PIBs/ml. DNA was resuspended in 30 ml of TE (10 mM Tris, 1 mM EDTA pH 8.0) and a sample labelled with ³²P by standard methods (Sambrook *et al.* 1989).

TABLE 1: Insect species used for testing with *Lymantria dispar* nuclear polyhedrosis virus.

Taxa	Life stage collected and source	Site	Diet for laboratory maintenance
BOMBYCIDAE <i>Bombyx mori</i> L	Commercially obtained ¹	Auckland	Mulberry leaves
GEOMETRIDAE <i>Epiphyne verriculata</i> (Felder & Rogenhofer)	Larvae on growing shoots of Cabbage Tree (<i>Cordyline australis</i>)	Lincoln	Growing tips of <i>C. australis</i>
<i>Pasiphila dryas</i> Meyrick	Larvae on native hebe (<i>Hebe parviflora</i> var. <i>arborea</i>)	Christchurch	<i>H. parviflora</i> var. <i>arborea</i>
HEPIALIDAE <i>Wiseana cervinata</i> Walker <i>Wiseana</i> sp.	Eggs collected from moths Eggs collected from moths	Templeton Lincoln	Modified soya bean diet ⁴ Modified soya bean diet
HYPONOMEUTIDAE <i>Nyctemera annulata</i> (Boisduval)	Larvae on groundsel	Christchurch	Groundsel
NOCTUIDAE <i>Chrysodeixis eriosoma</i> (Doubleday)	Larvae on Kowhai (<i>Sophora tetraptera</i>)	Lincoln, North Canterbury Christchurch	Lettuce, <i>S. tetraptera</i>
<i>Trichoplusia ni</i> (Hubner)	Larvae on Geranium (<i>Pelargonium quercifolium</i>)	Halswell	<i>P. quercifolium</i>
<i>Aletia cucullina</i> (Guenee)	Eggs collected from moths	Castle Hill	Lettuce, Plantain
NYMPHALIDAE <i>Danaus plexippus</i> L.	Larvae	Otago	Swan plants
OECOPHORIDAE <i>Hofmannophila pseudospretella</i> (Stainton)	Laboratory culture ³	Ruakura	Supplied diet
<i>Proteodes carnifex</i> Butler	Larvae on mountain beech (<i>Nothofagus solandri</i> var. <i>cliffortioides</i>)	Castle Hill	<i>N. solandri</i> var. <i>cliffortioides</i> leaves
PIERIDAE <i>Artogeia rapae</i> (L.)	Larvae on cauliflower	Lincoln	Cabbage, cauliflower, broccoli leaves
PYRALIDAE <i>Galleria mellonella</i> L.	Commercially obtained ²	Auckland	Farex (Boot Company, Wellington, New Zealand)
<i>Plodia interpunctella</i> (Hubner)	Commercially obtained ¹	Auckland	Supplied diet
TINEIDAE <i>Tineola bisselliella</i> (Hummel)	Laboratory culture ³	Ruakura	Supplied diet
TORTRICIDAE <i>Epiphyas postvittana</i> (Walker)	Commercially obtained ¹	Auckland	Supplied diet
<i>Cnephasia jactatana</i> (Walker)	Commercially obtained ¹	Auckland	Supplied diet
<i>Planotortrix excessana</i> ⁵ (Walker)	Commercially obtained ¹	Auckland	Supplied diet
<i>Ctenopseutis obliquana</i> ⁵ (Walker)	Commercially obtained ¹	Auckland	Supplied diet

¹ Obtained from HortResearch, Auckland, laboratory culture; ² Obtained from Biosuppliers, Auckland, commercial culture; ³ Obtained from Dr P. Gerard, AgResearch, Ruakura, laboratory culture; ⁴ L. Burgess, HortResearch, Auckland;

⁵ Kept non-communally due to their cannibalistic nature.

RESULTS

LdNPV had no effect on the 20 species of Lepidoptera, except in one experiment involving *P. dryas* (Table 2). A second experiment with *P. dryas* showed no effect of the virus. In no cases was virus detected in cadavers, either through visual examination or direct DNA probing (results not shown). For the slot-blot probing, serial dilutions of "Gypchek" could be detected to approximately 10,000 PIB. The virus sample sent to the US Forest Service (Hamden, USA) at the conclusion of the host experiments had an LC_{50} of 4.6×10^3 PIB/ml diet (LC_{95} 6×10^4 PIB/ml) against *L. dispar* larvae, compared to 9.0×10^3 PIB/ml diet for MR1-8 (John Podgwaite, pers. comm.). Thirteen of the twenty species undergoing viral bioassay as larvae completed their life cycle by emerging as healthy adults, which could then be identified.

TABLE 2: The effect of feeding 1.5×10^5 PIBs of *Lymantria dispar* NPV to Lepidoptera (inoculated larvae maintained at 20°C).

Taxa	Date dosed	Number tested (and deaths)		Significance of difference ¹
		Treated	Controls	
BOMBYCIDAE				
<i>Bombyx mori</i>	13.12.94 ²	10 (7)	10 (5)	ns
GEOMETRIDAE				
<i>Epiphyra verriculata</i>	7.12.94	26 (16)	25 (22)	ns
<i>Pasiphila dryas</i>	13.12.94	14 (8)	14 (0)	**
	6.1.95	7 (2)	9 (3)	ns
HEPIALIDAE				
<i>Wiseana cervinata</i>	9.1.95	2 (0)	1 (0)	ns
<i>Wiseana</i> sp.	24.1.95	48 (48)	48 (48)	-
HYPONOMEUTIDAE				
<i>Nyctemera annulata</i>	19.1.95	12 (0)	13 (0)	ns
NOCTUIDAE				
<i>Chrysodeixis eriosoma</i>	7.12.94 ²	1 (1)	1 (1)	-
	28.1.95	17 (1)	16 (0)	ns
<i>Aletia cucullina</i>	12.12.94	16 (5)	16 (4)	ns
	17.12.94	12 (0)	12 (0)	ns
<i>Trichoplusia ni</i>	6.1.95	7 (7)	9 (9)	-
NYMPHALIDAE				
<i>Danaus plexippus</i>	17.3.95	9 (1)	8 (0)	ns
PIERIDAE				
<i>Artogeia rapae</i>	11.1.95	17 (17)	20 (20)	-
	20.1.95	9 (3)	9 (2)	ns
PYRALIDAE				
<i>Plodia interpunctella</i>	13.12.94	24 (0)	24 (0)	ns
<i>Galleria mellonella</i>	12.1.95	10 (0)	9 (1)	ns
OECOPHORIDAE				
<i>Proteodes carnifex</i>	13.12.95	3 (2)	3 (3)	-
<i>Hofmannophila pseudospretella</i>	19.12.94	12 (0)	12 (0)	ns
TINEIDAE				
<i>Tineola bisselliella</i>	19.12.94	12 (4)	12 (3)	ns
TORTRICIDAE				
<i>Epiphyas postvittana</i>	18.1.95	23 (3)	24 (3)	ns
<i>Cnephasia jactatana</i>	18.1.95	22 (0)	20 (1)	ns
<i>Planotortrix excessana</i>	7.12.94	18 (3)	13 (5)	ns
<i>Ctenopseutis obliquana</i>	7.12.95	8 (2)	11 (6)	ns
	18.1.95	21 (2)	20 (1)	ns

¹ - indicates sample size too low or all died in both treated and control groups, invalidating the test; ns indicates not significant ($P < 0.5$); ** significant ($P < 0.1$).

² Inoculated with 1.5×10^4 PIBs twice due to taste aversion.

DISCUSSION

The object of exposing Lepidoptera to this pathogen was to determine whether LdNPV could infect New Zealand insects. Our original aim was to test against endemic Lepidoptera, but collecting and maintaining these proved difficult. Several specimens were collected but died before bioassays could be conducted. We collected larvae mainly in summer, as this is the period GM are larvae and the stage control measures would be required. To increase the number of species tested, we used any Lepidoptera available, including pest species. Other species were represented in the present study by less than 10 individuals (Table 2), so the test for infection lacked statistical power. There was no evidence in the bioassays that LdNPV could infect any of the tested Lepidoptera. These tests were conducted with greater than a LC_{95} dose of virus for GM and under constant laboratory conditions which could be expected to favour viral infection. The slot-blot examination also failed to detect any latent virus. This study supports previous findings that LdNPV does not attack hosts other than Lymantriidae (Barber *et al.* 1993 and references within). LdNPV has the advantage over the bacterial insecticide, Bt, of being specific to GM. For example, we treated nine *N. annulata* larvae with an equivalent of a field treatment of Delfin (Sandoz, Basle, Switzerland) and all died within 2 days. The lack of lymantriids in New Zealand also improves the chances that alternative hosts will not be found by the virus. Research has been conducted using both LdNPV and Bt for GM control in Germany, with promising results (J. Huber, pers. comm.), an approach which may have merit in New Zealand. However, further host range testing with combinations of pathogens would then be required. The research conducted both in this study and overseas suggests the virus would be a candidate for use in both an eradication campaign and against established populations of GM.

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