HOST RANGE TESTING OF A NUCLEOPOLYHEDROVIRUS OF THE LIGHTBROWN APPLE MOTH, *EPIPHYAS POSTVITTANA*

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ABSTRACT

Epiphyas postvittana nucleopolyhedrovirus (*Eppo*NPV) has considerable potential as a biocontrol agent or biopesticide for control of lightbrown apple moth, *Epiphyas postvittana*, a pest of horticultural crops in New Zealand, and a market access problem in export fruit. To obtain regulatory approval for *Eppo*NPV in either role *Eppo*NPV must not negatively impact on non-target organisms. In this study, eight non-target insect species from one hymenopteran and five lepidopteran families were inoculated with *Eppo*NPV at high titre (10^9 PIB/ml). Larval survival, growth rates, pupation and pupal weights were measured and larvae examined for *Eppo*NPV. Minor differences in one parameter were found in *Helicoverpa armigera* and *Cydia pomonella*, but no viral infection. Growth and survival were compromised in virus-fed individuals in only one species, *Tyria jacobaeae*. The majority of *T. jacobaeae* larvae had high microsporidial infections and *Eppo*NPV polyhedra were found in only one larva, suggesting a very low likelihood of field infectivity.

Keywords: *Epiphyas postvittana*, nucleopolyhedrovirus, host range, *Eppo*NPV.

INTRODUCTION

Epiphyas postvittana nucleopolyhedrovirus (*Eppo*NPV) is a baculovirus that attacks the light brown apple moth, *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae). The virus was isolated by the Department of Scientific and Industrial Research (DSIR) from laboratory colonies in the 1960s and was subsequently recorded in the field throughout New Zealand (Longworth & Singh 1980). In the mid 1970s, trials carried out by the DSIR at their research apple orchard in Nelson showed that virus extracted from larvae and applied as a dilute spray provided effective control of *E. postvittana* larvae (Longworth 1976). Subsequent work on the infectivity of *Eppo*NPV to the leafroller complex has shown that it is highly infective to *E. postvittana*, and has a range of infectivity to other members of the leafroller pest complex (Markwick et al. 2002).

Epiphyas postvittana is an important pest in New Zealand and is part of a complex of leafrollers that attack a number of horticultural crops, including apples, summerfruit, grapes and citrus, grown for export in New Zealand and Australia. Quarantine regulations in many countries (e.g. USA) have a nil tolerance of live leafrollers, or insect damage on imported fruit, and this can cause serious market access problems (Wearing 1991; Taverner et al. 1999; Lo & Murrell 2000; Mo 2005). *Epiphyas postvittana* in particular has also been implicated in the transmission of *Botrytis cinerea* in grapes (Bailey et al. 1997; Lo & Murrell 2000). Current control of this pest complex in export crops is achieved through Integrated Fruit Production (Kingston 2001; Wiltshire 2003) and Bt sprays in organic orchards. However, there are ongoing concerns regarding pesticide effects on natural enemies (Baker et al. 1994), withdrawal of the specific pesticides

from sale, minimum residue levels and ineffectiveness of Bt against leafrollers in apples (Wearing et al. 1994). Consequently all export fruit industries are looking at reducing pesticide use (Wiltshire 2003).

EppoNPV could provide effective control of the leafroller complex, and it could be used in combination with other existing methods and potentially address some of these concerns. However, in order to obtain approval for release of the virus as a biological control agent or its development as a biopesticide, Environmental and Risk Management Authority regulations require research demonstrating that the virus has no significant effect on non-target species. This paper describes a series of experiments testing for infectivity of *EppoNPV* to non-target species.

The insect species tested were selected for a variety of reasons. Codling moth (*Cydia pomonella*, Tortricidae) is also a pest in orchards and is closely related to *E. postvittana* but is from a different subfamily. The three members of the family Noctuidae – tomato fruit worm (*Helicoverpa armigera*), tropical army worm (*Spodoptera litura*) and soybean looper (*Thysanoplusia orichalcea*) – and the pine looper (*Pseudocoremia suavis*, Geometridae) are pests of a range of crops in areas where *E. postvittana* may also be found. Two beneficial insects were selected for testing, the honey bee (*Apis mellifera*, Apidae) and the cinnabar moth (*Tyria jacobaeae*, Arctiidae) that is a biocontrol agent for ragwort. The monarch butterfly (*Danaus plexippus*, Nymphalidae) was chosen for its anthropocentric value.

MATERIALS AND METHODS

Source of insects

Larvae were obtained from colonies kept at the Mount Albert Research Centre, or from eggs on foliage collected in the Auckland (monarch butterfly) or Wairarapa (cinnabar moth) regions. All eggs were sterilised before hatching and foliage was sterilised before feeding to larvae. Species that can be reared on artificial diets were treated with virus as neonates, while those fed on foliage were treated as 2nd instars, so that they were large enough to eat the treated leaf discs. Table 1 summarises the larval source and bioassay conditions.

TABLE 1: Insect species tested for susceptibility to *EppoNPV*, the origin of the insects, bioassay diets and temperatures, the age at inoculation and the days on which the larvae or pupae were weighed.

Insect species	Origin	Diet	Temp. (°C)	Age at inoculation	Weigh days
<i>C. pomonella</i>	Lab colony ¹	General purpose diet (Singh 1983)	20		
<i>P. suavis</i>	Lab colony ¹	Modified PAM diet (Barrington pers. comm), pine needles	20	Neonates	0, 7, 14, 21, P ³
<i>H. armigera</i>	Lab colony ¹	Lima bean diet (McManus & Burgess 1995)	23	Neonates	5, 12, P
<i>S. litura</i>	Lab colony ¹	Lima bean diet	23	Neonates	4, 11, P
<i>T. orichalcea</i>	Lab colony ²	Lima bean diet	23	Neonates	5, 12, 19
<i>T. jacobaeae</i>	Eggs from ragwort	Ragwort leaves	21	2nd instar	0, 7, 12, P
<i>D. plexippus</i>	Eggs from swan plant	Swan plant leaves	22	2nd instar	4, 11, P
<i>A. mellifera</i>	Brood frames from hives	Pollen food, 60% sucrose (Malone et al. 1998)	33	Newly emerged workers	–

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Source of virus

The source of *EppoNPV* is described in a leafroller infectivity study (Markwick et al. 2002). It has subsequently been passaged in *E. postvittana* twice, purified through a 5-step sucrose gradient and stored in water for 2 years at 4°C. This was tested against *E. postvittana* during the first host range bioassays in December 2005 to confirm viability of the *EppoNPV*.

Inoculation of the insects

All Lepidoptera were inoculated in 96 well microtitre plates. Virus-treated insects received a dose of 1×10^6 polyhedral inclusion bodies (PIBs) per larva suspended in water, while control insects just received sterile water. For those species for which an artificial diet had been developed, approximately 0.2 ml of diet was pushed into the bottom of the wells, and 1 µl of virus solution or sterile water was pipetted onto the diet and allowed to dry. Larvae were then left for 4-7 days on the treated diet (14 days in the case of *C. pomonella*), before being transferred onto uninfected diet. For species that were reared on foliage, 1 µl of virus solution was pipetted onto a 3 mm leaf disc and allowed to dry before being placed in the well with a disc of dampened filter paper (5 mm in diameter). After 24 h, larvae that had eaten the whole leaf disc were transferred to individual pottles with fresh uninfected foliage. There were three replicates containing at least 10 larvae per treatment for all species, except *T. orichalcea* where there was only one replicate. Mortality was recorded every 2-3 days, and larvae were weighed at weekly intervals, and as pupae.

Honey bees were collected just prior to, or immediately after, emerging from their cells, and maintained in groups of approximately 40 in cages with 60% (w/v) sucrose solution and water for 24 h. They were then force-fed a 2 µl droplet of solution consisting of 1 part virus at 10^9 PIBs/ml and 1 part 60% (w/v) sucrose solution before being returned to their cages and supplied with water and sucrose solution. For control insects the virus portion was replaced with sterile water. Pollen food was provided for the first 10 days after inoculation, and mortality was recorded three times per week. Three replicates of at least 38 bees per treatment were tested.

Analyses

Corrected percentage survival to pupation was calculated using Abbott's formula to determine control mortality (Abbott 1925). Survival of virus-inoculated and control insects was analysed by comparing plots of the Kaplan-Meier estimates of the Survivor function made using R version 2.4.1 (R Development Core Team 2006) in combination with the computer package 'survival' (Therneau & Lumley, undated). P-values are given for the approximate Chi-square statistic testing the difference between pairs of curves.

Growth was calculated as $\log_e$ final weight – $\log_e$ initial weight. The initial weight was taken on the day of inoculation and the final weight was the weight of the same larva on the last weigh day shown in Table 1. This calculation of growth rate can only be applied to the period of exponential growth (Markwick et al. 1995) and consequently the final weigh day varied from species to species depending on the length of their developmental period (Table 1).

Detection of PIB infection

A selection of the insects that died in each control and virus treatment was examined for the presence of *EppoNPV* for all species except *T. jacobaeae* where all deaths were checked. Live insects that appeared healthy were examined in species where there was no or low mortality (*H. armigera*, *S. litura* and *P. suavis*) and where growth of virus-fed larvae was significantly lower (*C. pomonella* and *T. jacobaeae*). Insects were dissected and a sample of their body tissue smeared on a slide. This was stained with Simple Giemsa stain and examined under a light microscope for the presence of PIBs. Where PIBs were detected visually, the presence of *EppoNPV* was determined by PCR using primers that target a gene unique to *EppoNPV* (Hyink et al. 2002).

RESULTS

All positive control *E. postvittana* larvae had died by day 11, consistent with results in Markwick et al (2002) and confirming that the *Eppo*NPV used in this study was viable.

Results in Table 2 show that corrected percentage survival to pupation of virus-inoculated larvae of all species except *T. jacobaeae* was not significantly different to control larvae.

TABLE 2: Survival (%) and growth rate of insect species inoculated with sterile water or *Eppo*NPV. The difference between control and virus-treated insects for these parameters is indicated by ns=not significant, *=P≤0.05 and **=P≤0.01.

Species	Number tested	% survival to pupation ¹	Mean larval growth rate ± SEM	
			Control	<i>Eppo</i> NPV
<i>C. pomonella</i>	143	86 ns	4.86 ± 0.04	4.43 ± 0.08 **
<i>P. suavis</i>	135	95 ns	5.84 ± 0.07	6.12 ± 0.08 *
<i>H. armigera</i>	116	98 ns	4.06 ± 0.04	4.00 ± 0.05 ns
<i>S. litura</i>	120	100 ns	4.56 ± 0.03	4.47 ± 0.04 ns
<i>T. orichalcea</i>	48	85 ns	3.95 ± 0.06	3.98 ± 0.06 ns
<i>T. jacobaeae</i>	120	59 **	4.76 ± 0.04	4.12 ± 0.16 **
<i>D. plexippus</i>	84	100 ns	4.36 ± 0.04	4.31 ± 0.05 ns

¹Corrected for control mortality.

Survival of honey bees treated with the virus was also not significantly different from the controls. The *T. jacobaeae* trials produced very variable results with high mortality (>30%) in both control and virus-fed larvae in one replicate and comparatively high mortality in virus-inoculated larvae in the other two replicates.

There was no significant reduction in growth rate in virus-inoculated compared with control larvae from any non-tortricid species, except *T. jacobaeae* (Table 2). In fact, growth in *P. suavis* treated with virus was significantly higher than the controls. The tortricid *C. pomonella* showed a small but significant reduction in growth in virus-inoculated larvae.

Larvae of all species pupated. Pupal weights of virus-inoculated females were not significantly different from controls in any of the species tested (Table 3). However, male *H. armigera* and *T. jacobaeae* pupae that had been treated with *Eppo*NPV were significantly lighter than control pupae.

TABLE 3: Pupal weights (mg) of insect species inoculated with sterile water or *Eppo*NPV. The difference between control and virus-treated insects for these parameters is indicated by ns=not significant, *=P≤0.05 and **=P≤0.01.

Species	Male		Female	
	Control	Virus	Control	Virus
<i>C. pomonella</i>	35.05	33.65 ns	40.46	39.64 ns
<i>P. suavis</i>	94.0	92.6 ns	111.2	105.8 ns
<i>H. armigera</i>	336.2	324.9 *	316.1	303.7 ns
<i>S. litura</i>	448.5	436.2 ns	456.6	461.0 ns
<i>T. jacobaeae</i>	172.4	161.1 *	184.6	180.2 ns
<i>D. plexippus</i>	1403	1466 ns	1347	1332 ns

Three larvae were found to contain PIBs when stained and examined under a light microscope. PCR analysis revealed that only one *T. jacobaeae* larva tested positive for *Eppo*NPV, while all others tested were negative. Many dead *T. jacobaeae* larvae examined under the microscope were found to have heavy microsporidian infections. No PIBs were found in larvae from the other species that died during the course of the trials.

DISCUSSION

The current study provides evidence that *Eppo*NPV does not infect insects from a range of non-tortricid lepidopteran families and does not affect survival of adult honeybees. Apart from the *T. jacobaeae* results the only effect observed was that virus-fed *H. armigera* produced significantly smaller male pupae than controls. However, larval growth rate in this species was not affected and no *Eppo*NPV PIBs were detected in the virus-fed larvae suggesting that a viral infection was not established.

The case of *T. jacobaeae* is less clear. Variation in survival between replicates prevented a direct correlation between mortality and virus inoculation. Further, many of the dead larvae had high microsporidian and bacterial infections despite egg and foliage sterilisation, and *Eppo*NPV was identified in only one larva. This suggests that viral infection was not the immediate cause of death in the vast majority of these larvae. Since the virus was found only in a single larva after inoculation with a dose greater than the LD₅₀ for *E. postvittana*, it would seem highly unlikely that an *Eppo*NPV infection would establish in this species in the field. Further testing would determine whether there is an interaction between a chronic disease such as microsporidia and *Eppo*NPV.

The tortricid species tested, *C. pomonella*, showed a significant reduction in larval growth rate when treated with the virus but this was not reflected in larval survival or pupal weights, and no *Eppo*NPV PIBs were detected. The effect on larval growth rates in this species could be beneficial as *C. pomonella* is also one of the major insect pests in New Zealand apple orchards. *Eppo*NPV has already been shown to have some infectivity to other tortricids besides *E. postvittana* (Markwick et al. 2002), and any efficacy against *C. pomonella* would extend its value as a biocontrol agent or biopesticide.

The current study suggests that the host range of *Eppo*NPV is likely to be restricted to members of the Tortricidae, although further testing, especially of the cinnabar moth, *T. jacobaeae*, would be required to confirm this. Specificity of a baculovirus to one insect family is not uncommon amongst baculoviruses. For example, *Mamestra brassicae*NPV is restricted to noctuids (Gröner 1986). It is well established that baculoviruses are confined to arthropods, and almost exclusively to insects (Gröner 1986, OECD 2002). However, there is considerable variation in the specificity of different viruses to insect species. For example, *Autographica californica*NPV is known to infect at least 33 species of insects (Gröner 1986), while *Euprocti chrysorrhoea*NPV, tested against 73 species, was shown to infect only the species it was originally isolated from (Cory et al. 2000). A study that tested *Lymantria dispar*NPV against 20 species of New Zealand Lepidoptera from 11 families also indicated a very narrow host range (Glare et al. 1995).

Baculoviruses are highly regarded as biocontrol agents or biopesticides due to their specificity to insects, and many countries are increasing their use of baculoviruses as biopesticides (Szewczyk et al. 2006). A baculovirus that targeted the leafroller complex would be a useful tool in the management of these insect pests in many horticultural crops in New Zealand, particularly pip-fruit, summer-fruit and grapes, and would benefit both IFP and organic fruit growers. It would undoubtedly find a ready market amongst Australian citrus and winegrowers as well.

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