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ORYCTES BACULOVIRUS INFECTIVITY FOR NEW ZEALAND SCARABS.

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ABSTRACT

This paper reports attempts to infect a range of insect species with Oryctes baculovirus. The following species of insects were tested: Heteronychus arator, Costelytra zealandica, Adoryphorus couloni, Pericoptus sp., Aphodius tasmaniae, Tenebrio molitor, Mythimna separata, Galleria mellonella, Inopus rubriceps, and sod webworms. The virus was infectious to varying degrees for all the scarabs tested except Aphodius tasmaniae. None of the non-scarabs tested could be infected.

INTRODUCTION

The control of the coconut palm rhinoceros beetle with Oryctes baculovirus is one of the best examples of microbial control of an insect pest (Huger 1966, Bedford 1980). In conducting these infectivity trials it was hoped we could emulate this success against one or more of New Zealand's scarab pests. This paper presents the results of these trials and discusses the possibility of using Oryctes baculovirus as a microbial control agent against New Zealand's two major scarab pests Heteronychus arator and Costelytra zealandica.

MATERIALS AND METHODS

Infected Oryctes rhinoceros beetles were kindly provided by the Samoan-German Crop Protection Project, Apia, Western Samoa. Oryctes baculovirus inoculum was obtained from the faeces of infected adult beetles. Infected beetles were placed in small plastic pots (diameter 55 mm) containing 2 ml 0.05M phosphate buffer (pH 7.2). Faecal material excreted into the buffer was collected every two days. Large particles were removed by low speed centrifugation (2,000 X g, 10 minutes). The supernatant was layered on a 10% (w/w) and 40% (w/w) sucrose gradient and centrifuged at 100,000 X g for 1 hour. Virus particles at the interface of the two sucrose solutions were collected, dialysed against 0.05M phosphate buffer to remove sucrose and then diluted with MM medium (Mitsuhashi and Maramorosch 1964) If the inoculum was to be injected it was also filtered (millipore 0.22 um) to render it aseptic and Gentamycin was added to a final concentration of 50 ug/ml.

Infection with Oryctes baculovirus was diagnosed using an indirect sandwich enzyme linked immunosorbent assay (ELISA) as described by Longworth and Carey (1980). To confirm the ELISA diagnoses samples of infected tissue were fixed, stained and embedded in resin for sectioning and examination by electron microscopy.

The insects tested were maintained for a maximum of four weeks before harvesting and analysis. H. arator adults and third instar larvae, C. zealandica third instar larvae, Adoryphorus couloni third instar larvae and Aphodius tasmaniae third instar larvae were maintained in sterilised peat and fed small pieces of carrot. C. zealandica first instar larvae were maintained on artificial diet (P. J. Wigley pers. comm.). Pericoptus sp. third instar larvae were maintained unfed in the same damp beach sand in which they were found (Karekare Beach, West Auckland). Tenebrio molitor adults were maintained on wholemeal flour, Mythimna separata on general purpose diet (Singh 1974), sod webworm on damp sphagnum moss, Galleria mellonella on its standard diet (Beck 1960), and Inopus rubriceps on damp filter paper.

The inoculation procedure used was governed by the size of the insect. Sod webworm and I. rubriceps were too small to inject or force feed so these insects were totally immersed in undiluted inoculum for ten minutes. First instar C. zealandica larvae had their heads only immersed in a drop of inoculum for 30 minutes. All the other insects were large enough to be injected. Each was injected with 2 ul of aseptic inoculum (see above) into the haemocoel, using a 30 gauge needle and the Isco model M microapplicator. Most of the large scarabs were also force fed 10 ul of inoculum using the same microapplicator and a blunt 30 gauge needle forced 3 to 4 mm down the foregut (Hutchins et al. 1984). Small pieces of the carrot diet were contaminated with 10 ul of the virus inoculum to try to infect C. zealandica, H. arator and A. couloni. Control insects were either fed, force-fed, or injected with distilled water.

RESULTS

The results of these infection trials are summarised in Table 1. With the exception of H. arator adults, all the successful trials showed a virus infection rate of between 60 and 100 per cent. Affected scarabs showed the typical symptoms of Oryctes baculovirus infection. The fat body lost its whiteness giving the larvae a glassy appearance and death occurred between 10 and 20 days after infection.

Despite using a wide variety of inoculation methods and highly concentrated inoculum we were never able to infect more than one in ten H. arator adults in any trial. The highest infection rates were achieved when the beetles were stressed by keeping them in the wells of plastic ice cube trays (well size 25 x 40 x 20 mm) without food or soil. All control insects were uninfected.

Table 1.

Infection trials with *Oryctes* baculovirus.

Insect	Stage	Inoculation method	% Infection	Number of insects
H. arator	adult	diet	<10%	>100
	adult	immersion	<10%	84
	1st instar	diet	72%	103
	3rd instar	diet	60%	20
	3rd instar	injection	85%	20
C. zealandica	1st instar	immersion	100%	13
	3rd instar	diet	0	25
	3rd instar	force fed	0	22
	3rd instar	injection	72%	44
Pericoptus sp.	3rd instar	force fed	0	6
	3rd instar	injection	100%	8
A. couloni	3rd instar	diet	0	11
	3rd instar	injection	96%	23
A. tasmaniae	3rd instar	force fed	0	15
	3rd instar	injection	0	13
T. molitor	larva	injection	0	24
M. separata	larva	injection	0	25
G. mellonella	larva	injection	0	20
I. rubriceps	larva	immersion	0	12
Sod webworms	larva	immersion	0	8

DISCUSSION

The effectiveness of *Oryctes* baculovirus control of *O. rhinoceros* is due to its ability to spread through the adult *O. rhinoceros* population and its pathogenicity, killing the *O. rhinoceros* adult within three weeks (Zelazny 1976). Overlap in generations of *O. rhinoceros* means there is always a pool of mobile and long-lived adults to keep the virus endemic.

H. arator, which has a long-lived and mobile adult population, might be controlled by a suitably pathogenic virus: however our results show that *Oryctes* baculovirus is not pathogenic enough for *H. arator* adults. *C. zealandica* adults were not tested as they are short lived.

Oryctes baculovirus is more pathogenic for H. arator larvae and to a lesser extent for C. zealandica larvae. The problem with such infections is that the larvae are relatively immobile so that transmission would be difficult. This would be alleviated if the virus could survive in the soil. However, Oryctes baculovirus, unlike most viruses in this group, is not embedded within a protective protein crystal (inclusion body) during its replication. Without this inclusion body the virus is rapidly inactivated in the soil. This means that any Oryctes baculovirus infection in a scarab larva would neither spread rapidly nor persist to infect another generation of larvae. If the Oryctes baculovirus could be genetically engineered to form its own protective inclusion body, it could survive in the soil and a valuable new microbial agent for New Zealand's scarab pests would exist. However Oryctes baculovirus, in its present form, is not suitable.

REFERENCES

Beck, S. D. 1960: Growth and development of the greater wax moth, Galleria mellonella (L.) (Lepidoptera: Galleriidae). Transactions of the Wisconsin academy of arts and letters. 49: 137-148.

Bedford, G. O. 1980: Biology, ecology and control of palm rhinoceros beetles. Annual review of entomology 25: 309-339.

Huger, A. M. 1966: A virus disease of the Indian rhinoceros beetle Oryctes rhinoceros (Linnaeus), caused by a new type of insect virus, Rhabdionvirus oryctes gen. n., sp. n. Journal of invertebrate pathology 8: 38-51.

Hutchins, R. F. N. ; Sutherland, O. R. W. ; Gnanasunderam, C. ; Greenfield, W. J.; Williams, E. M.; Wright, H. J. 1984: Toxicity of nitro compounds from L. pedunculatus to grass grub (Costelytra zealandica (Coleoptera: Scarabaeidae)). Journal of chemical ecology 10: 81-93.

Longworth, J. F.; Carey G.P. 1980: The use of an indirect enzyme linked immunosorbent assay to detect baculovirus in larvae and adults of Oryctes rhinoceros from Tonga. Journal of general virology 47: 431-438.

Mitsuhashi, Y.; Maramorosch, K. 1964: Leaf hopper tissue culture: Embryonic, nymphyl and imaginal tissues from asceptic insects. Contributions of the Boyce Thompson Institute. 22: 435.

Singh, P. 1974: Improvements in or relating to artificial dietary mixtures for insects. N. Z. Patent Application No. 174449.

Zelazny, B. 1976: Transmission of a baculovirus in populations of Oryctes rhinoceros. Journal of invertebrate pathology 27: 221-227.