An *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) Baculovirus Inoculum Derived from Tissue Culture

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ABSTRACT Large quantities of *Oryctes* baculovirus can be obtained in aseptic cell culture fluids using a recently established cell line derived from the scarab, *Heteronychus arator* (F.). Studies of thermal inactivation of the virus showed that it is extremely stable at 4°C and that at 28°C its stability is sufficient for extended field use.

THE USEFULNESS of *Oryctes* baculovirus in the control of the coconut palm rhinoceros beetle *Oryctes rhinoceros* (L.) is well documented (Huger 1966, Marschall 1970, Young 1974, Bedford 1980, Young and Longworth 1981). Zelazny (1976) showed that adult-to-adult contact during mating and preparation of the brood chamber is the major route of virus transmission. The virus may be introduced by releasing infected adults, which then spreads the virus throughout the population. The preferred method of inoculating adults before release is to add a small drop of virus suspension containing sucrose to the mouthparts of adult beetles, where it is readily imbibed (Bedford 1976).

The establishment of a continuous coleopteran cell line, DSIR-HA-1179 (HA cells) (Crawford 1982), which supports the replication of *Oryctes* baculovirus (Crawford 1981), has enabled large quantities of the virus to be produced in an aseptic, infectious, tissue culture fluid. In this form, virus is insulated from degradation by bacterial or fungal enzymes, and storage in a lightproof container prevents UV inactivation.

Because we considered thermal inactivation to be the only major storage problem, we examined the effect of a constant temperature of 4 and 28°C on the infectivity of the inoculum. We also tested whether a concentration of 5% (wt/wt) sucrose affected virus infectivity.

Materials and Methods

Virus was kept in Schneiders' tissue culture fluid (GIBCO), with and without sucrose, at 4 and 28°C. Samples from each treatment were taken at intervals over an 18-week period and assayed for infectivity by a tissue culture infectivity endpoint titration ($TCID_{50}$ assay). The assay was performed in 60-well Microtest II plates (Falcon Plastics) as previously described (Crawford and Granados 1982) and the $TCID_{50}$ titer was calculated by the statistical method of Reed and Muench (1938). The titer was expressed in infectious units (i.u.) by assuming that the probability of infection of a single well was according to a Poisson distribution, where $TCID_{50}$ i.u./ml = 0.69 × $TCID_{50}$ titer.

Results and Discussion

Virus infectivity was stable at 4°C (Fig. 1). Moreover, virus stored at 4°C for 1 year showed no significant change in infectivity ($P < 0.05$). At 28°C the infectivity titer decreased from 4×10^7 to ca. 4×10^4 i.u./ml after 18 weeks, an infectivity half-life of ca. 2 weeks. This result compares favorably with *Oryctes* baculovirus kept in a sawdust medium at 26°C, where a similar 1,000-fold decrease in infectivity occurred in 1 week (Zelazny 1972). The presence of sucrose, which makes the inoculum more palatable, did not affect virus infectivity.

Because New Zealand does not have an *O. rhinoceros* population in which to test this inoculum, we tested it on third stage larvae of the scarab *Heteronychus arator* (F.). The LD_{50} was 1.2×10^3 i.u. spread on a piece of carrot and fed to the larvae. It is likely, therefore, that virus infectivity in the inoculum could fall at least 100-fold and a droplet still have sufficient infectious virus for successful inoculation. In this case, the tissue culture fluid could be kept for up to 3 months at 28°C before it loses efficacy.

At present, virus must be kept frozen to retain infectivity and the only reliable method for transporting virus has been to ship via air the infected adult *O. rhinoceros*. Infectious tissue culture fluid could be packaged in small sealed ampules, sent via air mail and used on a daily basis. This would overcome a major logistical problem of providing active live virus in remote tropical regions. The best test of any new pathogen formulation is its usefulness in the field. Sufficient quantities of cell culture derived virus are available from the authors for any entomologist wishing to evaluate this inoculum against pest populations of *O. rhinoceros*.

Extensive safety tests of *Oryctes* baculovirus (Gourreau et al. 1979, 1981, 1982) have failed to

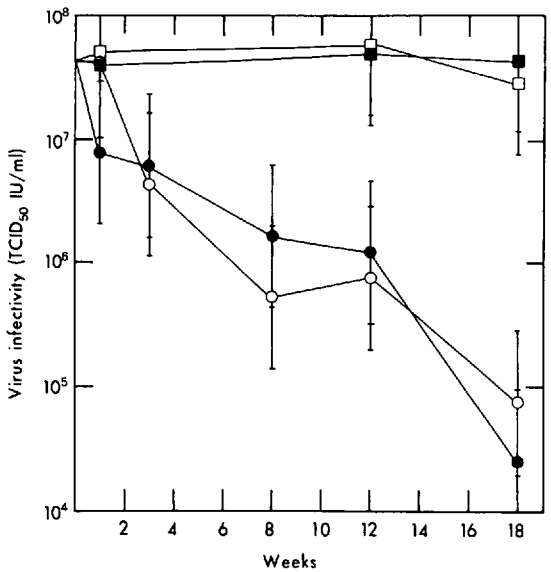

Fig. 1. Infectivity of *Oryctes* baculovirus in aseptic tissue culture medium stored at either 4 or 28°C and in the presence or absence of 5% (wt/wt) sucrose. □, 4°C, Without sucrose; ■, 4°C, with sucrose; O, 28°C, without sucrose; ●, 28°C, with sucrose. Error bars indicate the 95% CL of each infectivity estimate.

show any pathogenic effects of this virus for any of the vertebrates or vertebrate cell lines studied. The use of this virus in a sterile tissue culture fluid for insect mouthpart contamination is not hazardous.

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