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Persistence of *Oryctes* Baculovirus in Organic Matter

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ABSTRACT

Inactivation of *Oryctes* baculovirus in cattle dung was studied as a function of time. The infective half-life of *Oryctes* baculovirus was approximately for 5 days and total inactivation of the virus occurred on the 8th day in virus-cattle dung mixtures.

Key Words : *Oryctes* baculovirus, *Oryctes rhinoceros*, persistence

Oryctes baculovirus (OBV) has been successfully used in many South Pacific and Indian Ocean islands, Papua New Guinea and East Africa for the biological suppression of the coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) and *C. monoceros,* which are among the major insect pests of coconut and oil palm (Bedford, 1980, 1981; Gorick, 1980; Lomer, 1986; Purrini, 1989). OBV was first isolated by Huger (1966) from diseased *O. rhinoceros* grubs in Malaysia (OBV-MI). Subsequently OBV was found to occur in the wild populations of *O. rhinoceros* in the islands of Indonesia, Philippines (Zelazny, 1977a) and India (Mohan *et al.,* 1983). The Indian isolate of OBV (OBV-KI) has been used to control *O. rhinoceros* in Minicoy island, Lakshadweep (Mohan, 1990) and later introduced into the other islands of Lakshadweep, Andaman and Nicobar islands.

OBV is a non-occluded baculovirus and the primary site of infection in *O. rhinoceros* beetles and grubs is the midgut epithelium. Infected beetles excrete large amounts of OBV into the environment aiding dissemination of the disease. Farm yard organic heaps, decaying coconut trunks and stumps form the chief breeding sites for *O. rhinoceros.* Transmission of OBV disease to grubs in breeding sites occur by the visits of infected female (for egg laying, lasting for 3-4 days) and male beetles (for mating). The diseased beetles contaminate the organic matter with excreta containing OBV and grubs contract and succumb to the disease by feeding on the virus-contaminated organic matter. Beetles could also be infected *per os* during their stay in breeding sites containing OBV-killed larvae. Hence persistence of OBV in breeding sites is an important determinant in disease transmission. Generating information on the persistence of OBV-KI in the field was important in the study of the dynamics of OBV induced epizootics in introduced areas. This paper describes studies on the inactivation of OBV-KI in organic matter heaps.

MATERIALS AND METHODS

OBV-KI was isolated from the wild populations of *O. rhinoceros* beetles in Kerala state, India (Mohan *et al.,* 1983). The virus was routinely propagated either in beetles (Zelazny *et al.,* 1987; Mohan and Gopinathan, 1989) or in II or early III instar grubs of *O. rhinoceros* (Zelazny, 1972). Bioassays were performed with the crude virus preparation (CVP), prepared as follows: OBV infected midguts of II and III instar grubs were excised and homogenized in chilled phosphate buffer (50 mM, pH 7.6), clarified by centrifugation at 5000 x g for 10 min, and passed through a membrane filter (0.45 pore dia). The virus concentration was expressed as weight of infected tissue/ml. The CVP stock (500 mg infected tissue/ml) was stored at -20°C till use. The CVP of OBV-KI was bioassayed in II or early III instar *O. rhinoceros* grubs (Mohan *et al.,* 1985) and median effective dose (ED_{50}) was

calculated by Spearman and Karbers method (Hughes and Wood, 1986).

Two approaches were used to study the persistence of OBV in cattle dung heaps:

i) Five ml samples of filter-sterilized stock of OBV-KI (containing ~ 66 ED_{50} units/ml) were held in dialysis bags (molecular weight cutoff 12-15 x 10^6 Da) and buried in cattle dung heaps (moisture level 35-40%) at a depth of 25 cm. Dialysis bags were used to contain the virus, as this was a convenient method to prevent physical mixing of virus and cattle dung, and at the same time virus particles were exposed to the diffusible chemical constituents of cattle dung and to the temperature prevalent in the organic heaps. This method was useful in the sampling of virus from the dialysis bags. The temperature at a depth of 25 cm was recorded thrice a day. *O. rhinoceros* grubs had been observed to dwell at a depth of 20-30 cm in organic heaps of the kind used. Three heaps were used, each containing ten bags of OBV-KI and one of sterile phosphate buffer (control). Dialysis bags with OBV were removed on alternate days and ED_{50} of the virus sample was determined by bioassay in II instar grubs of *O. rhinoceros*.

ii) A volume of 80 ml of OBV-KI stock (500 mg/l) was mixed with 4 kg of cattle dung in earthen pots. The moisture was maintained at 40% level throughout the experiment. Samples of virus-treated organic matter (50g each) were drawn at periodic intervals (see Fig.1B) and the residual virus infectivity was bioassayed in II instar *O. rhinoceros* grubs. Ten early II instar grubs were exposed to samples from a single time-point and the experiment was replicated twice.

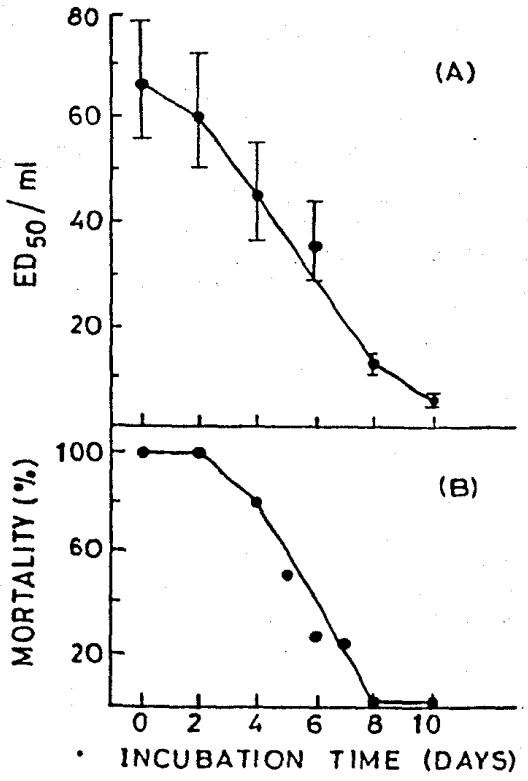

Fig. 1. Studies on persistence of *Oryctes* baculovirus in cattle dung

(A) Inactivation of virus with time in cattle dung heaps

(B) Inactivation in virus-cattle dung mixtures

RESULTS AND DISCUSSION

The average temperature of the cattle dung heaps at a depth of 25 cm ranged from 30-34°C. The maximum temperature of 34°C prevailed for 7-8 h in a day. Fig. 1A depicts the inactivation of OBV-KI in organic matter heaps in the field as a function of time. A steady decline in infective titer of OBV-KI in suspension was observed over a period of ten days (Fig.1A). The titer of infective virus on the 10th day was 7.6% (5.6 ED_{50}) of the initial titer of 66 ED_{50}/ml. In virus-organic matter mixtures (Fig.1B), the decline in infectivity almost followed a similar trend, except that inactivation could be detected only from the 4th day onwards and the rate of decline in infectivity was much faster. Total inactivation was observed

on the 8th day. The temperature of the virus-organic matter mixture ranged between 28-33° C.

The thermal inactivation point of OBV-KI in suspension is 56°C (Mohan *et al.*, 1985). The inactivation profiles of OBV-KI in organic matter point to the limited environmental stability of the virus in breeding sites. Infective half-life of OBV-KI was approximately for 5 days. Breeding sites have been seen to play an important role in disease transmission of OBV in introduced areas (Zelazny, 1976, 1977b; Young and Longworth, 1981). These sites act as reservoirs of OBV and grubs dwelling in it, and beetles visiting the breeding sites contract the virus infection. Based on the observed persistence of OBV it could be inferred that two conditions are necessary for the rapid spread of OBV disease in the pest population; (i) frequency of visits of beetles to breeding sites should be high enough to outstrip the inactivation of OBV in these sites, and (ii) the existence of multiple broods of *O. rhinoceros* in a breeding site. This helps to keep the site 'infective' for a long time. These ideal conditions for disease have been reported in many instances when OBV was introduced into South Pacific islands for the biocontrol of *O. rhinoceros* (Young, 1974, Zelazny, 1976, 1977b).

The inactivation of OBV-KI in the present studies was much slower than what has been reported for OBV-MI in virus-sawdust mixtures by Zelazny (1972) who observed that the proportions of infective OBV- MI remaining after one, two and four weeks of storage were 0.091, 0.027 and 0, respectively, of the initial dose inoculated into sawdust. Higher storage temperature and low moisture level were found to hasten inactivation (Zelazny, 1972). The slightly faster rate of inactivation of OBV-KI in virus-organic matter mixtures, as compared to virus in dialysis bags, could be due to direct contact of virus particles with inactivating factors from the microflora in the organic matter. OBV is exceptionally stable at low temperature. Crawford and Sheehan (1984) had reported negligible reduction in infectivity of OBV stored in sterile tissue culture fluid at 4°C for 1 year. Infectivity of OBV in tissue culture fluid at 28°C was found to be sufficient for field use in the tropics upto 3 months.

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