

10.

A NON-OCCLUDED BACULOVIRUS AGAINST THE RHINOCEROS BEETLE, A MAJOR PEST OF COCONUT AND OILPALM PLANTATION

Introduction:

A total of 547 insect and mite species is recorded on the coconut palm (Kurian *et al.*, 1979). The rhinoceros beetle *Oryctes rhinoceros* L. is one of the major pests which causes serious damage to palms of all ages. The adult beetle bores into the growing spear leaf cluster, spathes and petioles, and chews off the soft internal tissue which is pushed out as fibres through the holes. The leaf fronds when open, show a geometric 'V' shaped cut caused by the feeding of the beetle. Yield reduction by 10% occurs due to the drying of the inflorescence in spathe damage (Nair, 1986) accompanied by indirect loss caused by reduction in photosynthetic activity, and weakening of petioles. A method to assess the beetle damage has been worked out by Ramachandran *et al.*, (1963) and Zelazny (1979). The bore holes made by the beetle also serve as an entry point for another menacing pest, the red palm weevil and hosts of fungal pathogens. The beetle is stout and black in colour, 35-50 mm in length, 14-21 mm in breadth; with a cephalic horn, which is longer in males. The female beetles have dense reddish brown hairs on their pygidium. They lay eggs in the decaying organic matter, such as farmyard manure / cowdung heaps, dying and dead palm trunks, decaying stumps and compost pits so that grubs on hatching get plenty of food (Abraham, 1994). Recently it has been observed that coir-pith, accumulating as waste from local coir industries in Kerala, India has also become a favoured breeding site (Gopal and Sathiamma, 1998). The total life cycle of the pest is completed in an average of 171 days (Nirula 1955). In 1963, an investigation of diseases of *Oryctes rhinoceros* was conducted in Malaya, Fiji and Western Samoa. This survey resulted in the discovery of a Classical Microbial Control Agent *Baculovirus oryctes*

589.

- Workneh, F. and Van Bruggen, A.H.C. (1994) Microbial density, composition and diversity in organically and conventionally managed rhizosphere soil in relation to suppression of corky root of tomatoes. *Appl. Soil. Ecol.* 1: 219-230.
- Yuen, G.Y. and Raabe, R.D. (1979) Eradication of fungal plant pathogens by aerobic composting. *Phytopathol.* 69: 922.
- Zambolin, L. and Schenck, N.C. (1983) Reduction of the effects of pathogenic root infecting fungi on soybean by the mycorrhizal fungus *Glomus mosseae*. *Phytopathol.* 73: 1402-1405.
- Zehnder, G., Kloepper, J.W., Yao, C. and Wei, G. (1997) Induction of systemic resistance in cucumber against cucumber beetles (Coleoptera : Chrysomelidae) by plant growth-promoting rhizobacteria. *J. Econ. Entomol.* 90: 391-396.
- Zehnder, G.W., Yao, C., Murphy, J.F., Sikora, E.R. and Kloepper, J.W. (2000) Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth promoting rhizobacteria. *Biocontrol* 45: 127-137.

XXXX—XXX

Authors**George V.Thomas and S.R.Prabhu***Microbiology Section,**Central Plantation Crops Research Institute**Indian Council of Agricultural Research**Kasaragod 671 124, Kerala*

(*Rhabdionvirus Oryctes*) against the pest (Huger, 1966), and the infection was called locally as "The Malaya Disease". Later it was reported from the Philippines, Indonesia, Sumatra Island, Mauritius and West Kalimantan. Zelazny in 1981 mentioned about the presence of this virus in India, but Mohan and co-workers (1983) reported in detail about the baculovirus disease in 54.2 % natural population of rhinoceros beetles sampled from different locations in Kerala, India. The viral pathogen was then introduced in many other places and was proved to be a success. This resulted in it being documented as a landmark in the field of biological control of insect pests (Caltagirone, 1981)

The Virus

Huger (1966) placed this virus in a new genus and the pathogen was termed *Rhabdionvirus oryctes*. Later it was included as Subgroup C of Baculoviridae and was named *Baculovirus oryctes* (David, 1975). In 1991 Franckii put the occluded NPVs and GVs of Baculoviridae in Subgroup Eubaculovirinae and non-occluded ones like *Baculovirus oryctes* into Nudibaculovirinae.

Purified virus particles of *Baculovirus oryctes* (Zelazny *et al.*, 1985, Mohan and Gopinathan, 1989 b) consists of rod-shaped nucleocapsid (220-240 x 80-110 nm) surrounded by an envelope. The genome is double stranded super coiled DNA molecule of Mr 60-92 x 10⁶ daltons (Payne *et al.*, 1977). The melting temperatures of the DNA in 0.1 x SSC is 71.8°C corresponding to 43% G + C content and a total of 48 viral proteins have been identified from the Indian isolate (Mohan and Gopinathan, 1989 b, 1991). After detailed genomic characterisation of this isolate, Mohan and Gopinathan (1992) proposed the taxonomic status of a variant for OBV-K1, the Kerala isolate of *Oryctes baculovirus* (OBV), when compared to the Philippine isolate OBV-PV505.

Site of infection

Oryctes baculovirus gains entry into the host only through the ingestion of contaminated food. After gaining entry, it reaches the nuclei of midgut epithelium cells of larvae and adults (Payne, 1974), nuclei of cells of larval fat bodies (Huger, 1966), the haemolymph and also in the testicular and ovarian cells (Majumder and Jacob, 1993), where it replicates. In the 1st to 4th hour post infection period, virus adsorption to the plasma membrane and uptake in cytoplasmic vesicles occur. In 7th to 12th hour post infection period viral replication in the clear area of the hypertrophied nucleus occurs and finally sixteen and over hours later virus

release from plasma membrane takes place (Crawford and Sheehan, 1985).

Diagnostic symptoms of OBV disease on *O. rhinoceros*:

The grub and the adult stages of *Oryctes* are infected by this pathogen.

Effect on grubs:

Infected grubs become lethargic and stop feeding. They come to the surface of the feed, the abdomen becomes turgid and glassy with chalky white spots. As the virus multiplies the fat body disintegrates and the haemolymph content increases. The midgut also becomes devoid of food and gets filled with white mucoid fluid which causes translucency of the midgut line dorsally when seen against bright light (Huger, 1966). Increase in turgor pressure in abdomen sometimes leads to extrusion of rectum. The infected grubs die within 15-20 days, and do not pupate. The period of lethal infection depends on the larval stage and surrounding temperature. High temperature at about 32°C speed up the death (Zelazny, 1972). The order of susceptibility among three instars is I>III>II (Mohan *et al.*, 1985 a). On dissection of the infected grub the swollen midgut filled with white colour fluid is seen.

The healthy grubs on the other hand are active, feed vigorously, remain beneath the feed and present a clear dark midgut line on dorsal side. When dissected midgut filled with black colour feed is seen.

Effect on adults:

The adults also become lethargic and stop feeding. When dissected, the midgut filled with white mucoid fluid is seen. In contrast, the healthy beetles have a very thin, brown midgut containing very little clear brownish fluid.

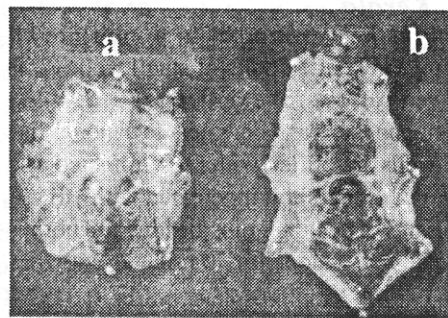


Fig. Baculovirus-infected grub (a) showing white fluid filled midgut whereas healthy one (b) shows black food material inside.

The longevity of the infected adult comes down to 25 days (cf 75) and fecundity of the females to one or two eggs (cf 15 eggs) as compared to healthy ones (Zelazny, 1973 a). The infected beetles also excrete virus contaminated faeces upto 0.3mg/day and thus spread the pathogen in nature (Monsarrat and Veyrunes, 1976).

In addition, the infection causes changes in the haemocyte count, particularly, that of granular cells and plasmocytes, and protein, amino acids and sugar content of the host (Vincent *et al.*, 1988; Biju *et al.*, 1993).

Detection of the virus in the insect body:

The presence of the virus in the infected host can be detected by the following methods:

Smear method:

Air dried smears of midgut fluid and midgut epithelial cells, fixed in methanol for 2-5 min, stained in 3% Giemsa stain for 45-60 min and finally rinsed in distilled water (Zelazny, 1973 b) shows large clumps of cells with purple stained hypertrophied nuclei (18-28 μm) and sparse blue cytoplasm. Also in many cases a homogeneously stained deep pink circular band along the periphery of the nucleus is seen. In contrast, the number of free cells in the smears of healthy midgut is significantly less, with considerably small pink coloured nuclei (7.5 - 12. μm) (Mohan *et al.*, 1983).

Immuno - osmophoresis (IOP):

This technique is used as a rapid method for the detection of baculovirus. A slight modification of the procedure by John (1965) is followed. A volume of 4.5ml of 0.8% warm "oxoid" agar in 0.045 M phosphate buffer, pH 7.4, containing 0.1% sodium azide is pipetted on to a-Formvar 15/95 E (0.2% W/V in chloroform) coated microscope slide. To make the agar film firm, the slide is kept overnight in a moisture chamber under refrigeration. Using a gel cutter, pairs of small wells (2mm, dia) are cut 1 cm apart in 2 or 3 vertical rows. The vertical rows of wells on the cathodic end constitute the antigen wells and ones near the anodic end the antiserum wells. The antigen wells are filled with OBV infected midgut aspirates, and antiserum obtained from rabbit inoculated with OBV in the opposite wells. Midgut aspirates of healthy grubs/beetles and normal rabbit serum serves as control. The IOP is done for 70 minutes with the current of 12-15 mA and voltage of 10 V/cm. Phosphate buffer, 0.045 M, pH 7.4 used for agar gel is also used as an electrolyte in the buffer tank. A precipitin line of growing intensity with time is noticed after the run. However, maximum intensity is obtained after 30 minutes incubation at room temperature (Mohan and Pillai, 1983).

An Enzyme linked immunosorbent assay (ELISA) for detecting OBV has also been reported (Longworth and Carey, 1980; Mohan and Gopinathan, 1989 a).

Bio-assay :

Midgut of diseased beetle or grub is homogenised in minimum volume of phosphate buffer (0.001 M, pH 8.5) containing antibiotics (Streptomycin 0.5g/l, Aureomycin 0.3g/l, Chloramphenicol 0.3g/l) and clarified by centrifugation. This homogenate is fed orally to healthy grubs (5/container) using a syringe and are maintained in moist autoclaved cowdung or cowdung-sawdust mixture (2:1 w/w), and observed for the appearance of characteristic translucency of the thoracic region and death due to OBV infection within five weeks. The control samples should be alive during this period. If even one inoculated grub in a test group exhibits typical disease symptoms, it is considered to be bio-assay positive (Mohan *et al.*, 1983; Zelazny, 1978).

Electron microscopy:

One millimetre thick midgut slices are fixed in 2% glutaraldehyde in 0.2 M, pH 7.2 phosphate buffer and stained with 1% osmium tetroxide and finally embedded in "Epon-araldite". When examined under EM presence of bacilliform virus particles of 220-240 x 80-110 nm, with each nucleocapsid envelope in distinct membrane is observed (Fig-6) (Mohan *et al.*, 1983; Mohan and Gopinathan, 1992).

Analysis of beetle excreta :

OBV infected beetles are kept in plastic container with 5ml of phosphate buffer saline (PBS) (0.01 M, pH 7.0, NaCl 0.85%), just enough to be in level with the distal end of abdomen of the beetles. The buffer contains Streptomycin, 250; Penicillin, 200; Oxytetracyclin, 100 mg/l to prevent bacterial contamination. The faecal matter collected is centrifuged at 500 rpm for 10 minutes. The sediment is resuspended in 0.2ml PBS and observed by 3% Giemsa staining for the typical hypertrophied nuclei (Mohan *et al.*, 1985 b).

Thus, the diagnosis of the OBV infection by observing the primary external pathological symptoms is very important in the case of grubs and adults. To confirm the disease smear test followed by IOP prove to be ideal in view of high degree of reliability, rapidity and economy. For the bioassay, considerable precaution is to be taken to prevent cross contamination between test groups. The other methods mentioned are highly technical, time consuming and involve expensive chemicals and instruments.

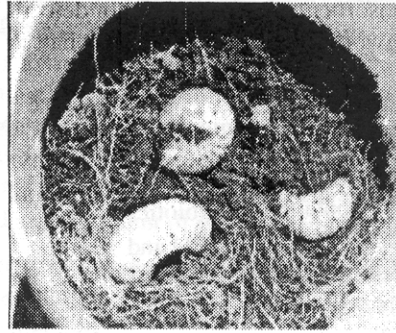


Fig 2
Baculovirus - inoculated rhinoceros grubs maintained in laboratory, in coir waste.

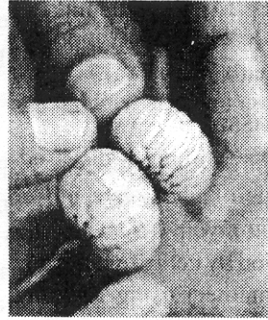


Fig. 3
External baculovirus disease symptoms (Upper-disease grub; Lower healthy grub.)

Production, maintenance and storage of the viral inoculum :

Production and maintenance of the virus is done by propagating it in live grubs/beetles of the *Oryctes*. Eight virus killed grubs are homogenised and mixed with sterilised sawdust or cowdung or cowdung-sawdust mixture filled in a plastic box of 61 x 27 x 41 cm. Some 100-150 healthy grubs are released into the box and allowed to feed for 5-7 days. They are then transferred to similar box with sterilised feed until they develop the symptoms and die (Bedford, 1976). The authors of this article have found an alternative feed in the form of coir-pith/ waste which is available in plenty in Kerala from coirbased industries. The sterilised coir waste serves as a better feed than cowdung because of lesser bacterial contamination in the grubs when maintained in it (Gopal and Sathamma, 1998). Alternatively, the infected midgut of beetle/grub can be excised, homogenised (in few ml of phosphate buffer saline, pH 7.0) and orally fed to the healthy grubs or beetles and maintained in the sterilised feed as mentioned elsewhere in the article for the production and maintenance of the viral inoculum.

Maintenance of the virus can also be done on *O. rhinoceros* cell culture (Quiot *et al.*, 1973), in *Spodoptera frugiperda* and in *Aedes albopictus* (Kelly, 1976) cell lines which has been disputed (Crawford, 1981). Cell lines derived from *Heteronychus arator* F. support the replication of this virus (Crawford, 1982; Crawford and Sheehan, 1985).

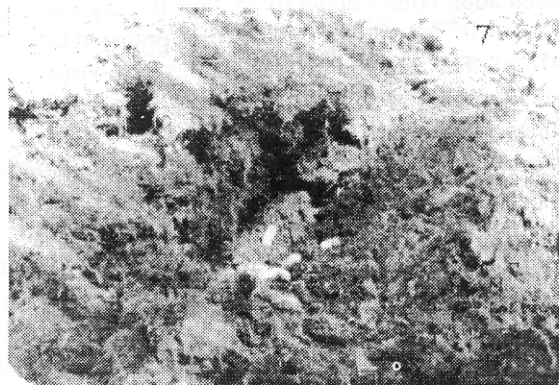


Fig. 4. Rhinoceros grubs breeding in coir waste dump.

A production method currently being used by the Philippine Coconut Authority, wherein, virus harvested from cell lines is used to infect beetles, and the midgut from 5 beetles is homogenised on ice, suspended in 2ml buffer (50 mM Tris-HCl, pH 8.5, 100 mM NaCl, 1 mM EDTA and 10 mM Na_2SO_3) spun for 10 min at 5000 x g and loaded on 15 mm wide chromatography column packed with controlled pore glass (Sigma, 700 A pore size, 120-200 mesh size) to a height of 80cm. The virus suspension is eluted out from the column with 50 mM Tris-HCl buffer. The first clear 60 ml is discarded and the next 20 ml of turbid eluate containing the virus is collected. Sucrose is added to reach a concentration of 10% w/v. This is filtered through 0.22 μm Millipore filter and dispensed in 2 ml lots of sterile serum vials. This can be stored at room temperature for several weeks, is also stable when refrigerated. Whenever needed the inoculum can be fed orally to the beetles for infection (Zelazny *et al.*, 1987).

The virus packed cadavers can be stored indefinitely at -40°C (Bedford, 1976). Generally it is inactivated within two weeks under normal conditions as virus packed cadavers, the virus suspension is also inactivated at 70°C for 10 min, or by 1% solution of formaldehyde or dettol (Zelazny, 1972).

Transmission of the virus :

The virus is perpetuated in nature by infected host. It is transmitted mostly during mating, possibly when the healthy partners contacts by mouth virus defecated by the infected partners or similarly when healthy and infected beetles feed together. The beetles also pass infection to healthy grubs when they visit breeding sites (Zelazny, 1976). This mode of trans-

Fig 5



Fig. 6

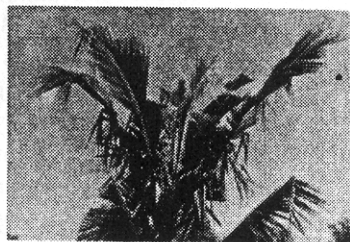


Fig. 7

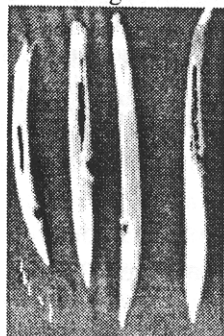
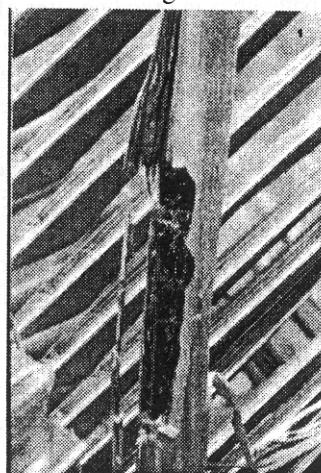


Fig. 8



- Fig. 5 A coconut palm infested by rhinoceros beetle (*Oryctes rhinocero*)
 Fig. 6 Geometric cuts of coconut leaves made by rhinoceros beetle
 Fig. 7 Spathe tissue chewed up by the beetle, thus affecting the
 inflorescence
 Fig. 8 Damage in newly emerged spindle portion

mission is supported by a mathematical model where rates of 6 different transmission pathways of the baculovirus were estimated and transmission from infected to susceptible feeding adults was proved to be the dominant route (Hochberg and Waage, 1991). Infected beetles excrete viral contaminated faecal matter from 3rd and 9th day after infection into the surrounding (Mohan *et al.*, 1985b) and the rate of spread of the virus in the field is estimated to be about 1 km/month (Jacob, 1996).

No virus infections occur in grubs hatching from egg surface-contaminated with the virus and grubs hatching from eggs laid by virus-infected females (Zelazny, 1976).

Application of the virus:

The simplest, most economical and direct method of virus dissemination is by the release of virus-infected adults (Bedford, 1981). Healthy beetles collected from field are allowed to wade through virus inoculum contained in a basin for 30 min. The inoculum is prepared by homogenising 1 g infected larval tissue in 1 litre of phosphate buffer (0.05 M, pH 8.0) which amounts to 31.6 LD₅₀ dose and 3% sucrose. The beetles can also be inoculated by the purified viral inoculum prepared as mentioned by Zelazny *et al.*, (1987). After swim treatment where the beetles suck in some virus they are confined together for 12 to 24 hr, and released after dusk at the rate of 15 inoculated beetles per hectare.

This method eliminated the disadvantages of other methods of application where compost heap or split coconut log heap is prepared artificially and inoculated with virus-killed larval tissues (Bedford, 1981). The infected beetles disperse widely before death, spreading the disease directly into the wild population, contaminating breeding sites which may contain larval broods and other beetles, and the palm crowns.

Other hosts of the virus :

A series of related dynastine which are agricultural pests also proved to be more or less susceptible to the OBV, e.g., *Oryctes nasicornis*, *O. monoceros*, *O. boas* (Huger, 1966; Julia and Mariau, 1976 and Purrini, 1989). *Scapenes australis grossipunctatus* (Bedford, 1973), *Papuana uninodis* (Zelazny *et al.*, 1988) and *Xylotripes gideon* (Dangar *et al.*, 1994). *Sternochetus mangiferae*, a curculionid pest of mango nut is described to be infected by a virus, showing similar symptoms as that of OBV (Shukla *et al.*, 1984) and contradictory observations have been reported in the case of pathogenicity to cashew stem and root borer by the OBV (Bakthavatsalam and Sundararaju, 1990).

Safety Test:

In a safety test conducted in France, no pathogenicity was observed on eight tissues; two human and two pig cell cultures, and one each from mouse, hamster, fish and calf. Same results were obtained when observed in living inoculated mice, or in other organs of mice up to 60 days after inoculation (Anonymous, 1973; Gourreau *et al.*, 1979; 1982). There is also no evidence of this virus as being infective on the natural enemies of its homologous pest.

Impact of virus release:

The impact of virus release can be assessed directly by observing the fresh leaf, spathe or inflorescence damage on coconut palms, trapping of the beetles and diagnosing for OBV disease and also by recording the population of the host at breeding sites (site occupancy test). The result can be had only when the same observations are recorded before the release of the virus. Detailed methods of survey have been described by Young (1974), Bedford (1976) and Mohan *et al.*, (1989).

Drastic reduction in beetle population and crop damage has been reported in South Pacific Islands, Fiji, Mauritius, Seychelles and Papua New Guinea where this pathogen was released to suppress the *Oryctes* population (Marschall, 1970; Hammes, 1971; Young, 1974; Bedford, 1976 and Gorick, 1980). Successful performance of baculovirus in Indian scenario in Minicoy Island, Lakshadweep (Mohan *et al.*, 1989), in Androth Island, Lakshadweep (Pillai, pers comm) Chitilappily of Thrissur in Kerala (Sathiamma, pers comm) and in Andaman Islands (Jacob, 1996) is summarised in the Tables 1, 2, 3 and 4 respectively. In oilpalm plantations of Kerala also this virus has proven itself to be a good biological control agent of the rhinoceros beetle (Dhileepan, 1994).

Future lines of investigation:

In the light of various investigations carried out on *Oryctes* baculovirus, following areas have to be probed in depth. Intensive efforts have to be taken up on the development of recombinant baculovirus of *Oryctes*. This line of work is being already pursued on other baculoviruses infecting lepidopteran pests (Bonning and Hammock, 1996). The method of application of OBV and its storage are two aspects, which require refinement. The formulation of commercial product is a highly desirable step required for the wide popularisation of this viral pathogen.

In India, a thorough survey in all the coconut growing tracts to spot out the occurrence of this viral pathogen in the natural population of the beetles is required so that wherever its introduction is required it can be

taken up. Work in Philippines have indicated a significant positive correlation between baculovirus incidence and rainfall but, a marginal negative correlation with temperature (Villacarlos and Betonio, 1990). Investigations currently being carried out by the authors in Kerala, India indicate the low incidence of the pathogen in the nature at least during certain seasons (Unpublished). Hence a detailed survey is required on the host-pathogen interaction in nature so that augmentative release of the biocontrol agent may be taken up whenever warranted.

IPM strategies adopted for control of rhinoceros beetle in India has various components. The influence of these components, especially chemicals on the viral pathogens in breeding sites of the pests is to be studied. The sustainability of infection in natural population of the pest in various stages of its life cycle requires a thorough investigation for which transovarial and transtadial transmission of the pathogen is to be ascertained.

Being a classical microbial control agent reported from many geographical zones in South East Asia and Pacific region, improvement of the pathogen by selection and development of a more virulent strain can be thought of and this process may help to tide over situations of the host insect developing resistance to the pathogen as it is suspected at least in some places (Zelazny *et al.*, 1989).

Table 1. Impact of the introduction of baculovirus disease into *O.* rhinoceros population in Minicoy (India), on crop damage in experimental plots.

	Means(%) of experimental plots		
	Leaf damage	Spathe damage	Spindle damage
Pre-release			
April 83	55.83	25.90	29.56
Post-release			
Jan 84	45.43	8.86	8.27
Nov 84	25.57	1.95	1.84
Sept 85	12.89	1.61	2.90
CD 5%	5.40	6.14	9.72
CV (%)	9.36	16.73	31.32
SEM	1.75	1.88	3.16

Table 2. Performance of *Oryctes baculovirus* in Androth Island of Lakshadweep in India

Period of survey	Leaf damage	Spathe damage	Fresh spindle damage	OBV incidence
Pre-rel (Apr 88)	55.0	7.3	23.5	0.0
Post-rel (Dec 88)	43.0	3.0	15.7	35.6
Post-rel (Jan 90)	13.5	3.2	5.9	60.6

Table 3. Effect of re-release of baculovirus in an already infected contiguous area at Chittilappilly, Thrissur, India.

	% damage			
	Palms	Leaf	Spathe	Spindle
Pre-release				
July 1989	100	34.4	12.5	68.18
Post-release				
Feb 1990	64.29	23.76	0.00	50.00
July 1990	64.71	27.07	0.00	17.65
March 1991	89.47	33.96	6.52	0.00
Aug 1992	22.73	6.66	0.00	0.00

Table 4. Percentage of virus infection in beetle population at Sipighat, Andamans (India)

Observations	Per cent virus infection
Pre-release 1987 (March)	0%(n=81)
Post-release 1987 (December)	53%(n=53)
1988 (Dec)	77%(n=17)
1989-1991	61%(n=18)

n=number of beetles observed

REFERENCES

- Abraham C.C. (1994) Pests of coconut and arecanut. In: *Advances in Horticulture*. Vol.10 Plantation and Spice Crops. Part 2. K.L.Chadha and P.Rethinam (Eds) Malhotra Publishing House, New Delhi- p.715-716.
- Anonymous (1973) The use of virus for the control of insect pests and disease vectors. *FAO/WHO Tech. Report Ser.* 531, pp.48.
- Bakthavatsalam N. and Sundararaju D. (1990) Pathogenicity of *Oryctes* Baculovirus to cashew stem and root borer. *J.Biol. Cont.* 4(2), 127-129.
- Bedford G.O. (1973) Experiments with the virus *Rhabdionvirus oryctes* against the coconut palm rhinoceros beetle *Oryctes rhinoceros* and *Scapanes australis grossipunctatus* in New Guinea. *J. Invertebr. Path.* 22, 70-74.
- Bedford G.O. (1976) Use of virus against the coconut palm rhinoceros beetle in Fiji. *PANS*.22, 11-25.
- Bedford G.O. (1981) Control of the rhinoceros beetle by baculovirus. In *Microbial control of pests and plant diseases 1970-1980*. Burges H.D(ed). Academic press. London. p. 409-426.
- Biju Babjan, Sudhadevi K., Dangar T.K., Sathiamma B. and Pillai G.B. (1993) Effect of Baculovirus infection on carbohydrates, protein and amino acid levels, and protease activity in haemolymph of *Oryctes rhinoceros* grubs. *J. Plantn. Crops* 21(2), 88-96.
- Bonning B.C. and Hammock B.D. (1996) Development of recombinant Baculoviruses for insect control. *Annu.Rev.Entomol.* 41, 191-210.
- Caltagirone L.S. (1981) Landmark examples in classical biological control. *Annu. Rev. Entomol.* 26, 213-232.
- Crawford A.M. (1981) Attempts to obtain *Oryctes* baculovirus replication in three insect cell cultures. *Virology* 112, 625- 633.
- Crawford A.M. (1982) A coleopteran cell line derived from *Heteromychus arator* (Coleoptera: Scarabaeidae). *In vitro* 18, 813- 816.
- Crawford A.M. and Sheehan C. (1985) Replication of *Oryctes* baculovirus in cell culture : viral morphogenesis infectivity and protein synthesis. *J.Gen. Virol.* 66, 529-539.
- Dangar T.K., Solomon J.J. and Pillai G.B. (1994) Infection of the coconut palm beetle, *Xylotrupes gideon* (Coleoptera:Scarabaeidae) by a nonoccluded baculovirus. *Z. Pflankranh. Pflschutz.*, 101(6), 561-566.

- Lakshadweep by *Oryctes* baculovirus impact on pest population and damage. *J. Plantn Crops* 16 (Suppl.), 163-170.
- Monsarrat P. and Veyrunes J.C. (1976) Evidence of *Oryctes* virus in adult feces and new data of virus characterizations. *J. Invertebr. Path.*, 27, 387-389.
- Nair M.R.G.K. (1986) Insects and mites of crops of India. Indian Council of Agricultural Research (ICAR). New Delhi, India. p.83.
- Nirula K.K. (1955) Investigations on the pests of coconut palm. Part II. The *Oryctes rhinoceros*. *L. Indian Cocon. J.* 8(40), 161-180.
- Payne C.C. (1974) The isolation and characterization of a virus from *Oryctes rhinoceros*. *J. Gen. Virol.* 25, 105-116.
- Payne C.C., Compson D. and De Looze S.M. (1977) Properties of the nucleocapsids of a virus isolated from *Oryctes rhinoceros*. *Virology*. 77, 269-280.
- Purrini K. (1989) *Baculovirus oryctes* release into *Oryctes monoceros* population in Tanzania with special reference to interaction of virus isolates used in our laboratory infection experiments. *J. Invertebr. Path.* 53, 285-300.
- Quiot J., Monsarrat P., Meynadier G., Croizier G. and Vago C. (1973) Infection des cultures cellulaires de coleopteres par le virus *Oryctes*. *Comptes rendus hebdomadaires des seancea de l' Academie des sciences, serie D.* 276, 3229-3231.
- Ramachandran C.P., Kurian C. and Jacob Mathew. (1963) Assessment of damage to coconuts due to *Oryctes rhinoceros*. *L. Nature of damage caused by the beetle and factors involved in the estimation of loss. Indian Cocon. J.* 17(1), 3-12.
- Shukla R.P., Tandon P.L. and Singh S.J. (1984) Baculovirus a new pathogen of mango nut weevil *Sternochetus mangiferae* (F.) (Coleoptera:Curculionidae). *Curr.Sci.* 53, 593-594.
- Villacarlos L.T. and Betonio. P.A. (1990). Incidence of baculovirus and green muscardine fungus in the larval population of rhinoceros beetle in Baybay, Leyte, Philippines. *Philippine J. Cocon. Stud.* XV(1), 21-23.
- Vincent V.M.J, Ali S.H. and Lakshamanan M. (1988) Baculovirus infection of *Oryctes rhinoceros*. Effect of virus on the total and differential haemocyte counts. *J. Biol. Cont.* 2(1), 29-32.
- Young E.C. (1974) The epizootology of two pathogens of the coconut palm rhinocerosbeetle. *J. Invertebr. Path.* 24:82-92.
- Zelazny B. (1972) Studies on *Rhabdionvirus oryctes* I. Effect on larvae of *oryctes rhinoceros* and inactivation of the virus. *J. Invertebr. Path.* 20:235-241.

- Zelazny B. (1973a) Studies on *Rhabdionvirus oryctes*. II. Effect on adults of *Oryctes rhinoceros*. *J. Invertebr. Path.* 22, 122-126.
- Zelazny B. (1973b) UNDP/FAO Rhinoceros Beetle Project. *Annu. Report.* p, 62-63 Apia.
- Zelazny B. (1976) Transmission of a baculovirus in populations of *Oryctes rhinoceros*. *J. invertebr. Path.* 27, 221-227.
- Zelazny B. (1978) Methods of inoculating and diagnosing the baculovirus disease of *O. rhinoceros*. *FAO Plant Prot. Bull.* 26, 163-168.
- Zelazny B. (1979) Loss in coconut yield due to *Oryctes rhinoceros* damage. *FAO Plant Prot. Bull.* 27(3), 65-70.
- Zelazny B. (1981) India- presence of the baculovirus of *oryctes rhinoceros*. *FAO Plant Prot. Bull.* 29(3/4), 77-78.
- Zelazny B., Alfiler A.R. and Crawford A.M. (1987) Preparation of baculovirus inoculum for use by coconut farmers to control rhinoceros beetle (*Oryctes rhinoceros*). *FAO Plant Prot. Bull.* 35(2), 36-42.
- Zelazny B., Alfiler AR. and Lolong A. (1989) Possibility of resistance to a baculovirus in populations of the coconut rhinoceros beetle (*Oryctes rhinoceros*). *FAO Plant Prot. Bull.* 37(2), 77-82.
- Zelazny B., Alfiler A.R. and Mohamed N.A. (1985) Glass permeation chromatography for purification of the baculovirus of *Oryctes rhinoceros* (Coleoptera : Scarabaeidae) *J. Econ. Entomol.*, 78(4), 992-994.
- Zelazny B., Avtar M.L., Rajendrasingh and Malone L.A. (1988) *Papuana uninodis*, a new host for the baculovirus of *Oryctes*. *J. Invertebr. Path.* 51(2), 157-160.

Murali Gopal and Alka Gupta

Central Plantation Crops Research Institute, Regional
Station, Kayangulam,
Krishnapuram – 690533, Kerala, India.