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BACULOVIRUS DISEASE IN *ORYCTES RHINOCEROS* POPULATION IN KERALA*

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ABSTRACT

A baculovirus disease was found in *Oryctes rhinoceros* beetles in Kerala. The symptoms conformed to those reported from South East Asia and the South Pacific Islands. The disease was diagnosed and confirmed by visual symptoms of midgut, smear of midgut contents, immuno-osmophoresis, bioassay in grubs and beetles and electron microscopy of midgut sections of the beetle. The smear test and immuno-osmophoresis were found to be ideal diagnostic methods for routine screening. The bacilliform baculovirus particles measured 215-260 × 77 - 108 nm.

INTRODUCTION

The rhinoceros beetle, *Oryctes rhinoceros* (L.), is one of the major pests of the coconut palm in India, South East Asia and the Pacific Islands. A virus disease of the pest was recorded by Huger (1966) in Malaysia and was subsequently introduced into several of the South Pacific Islands to control the pest population (Marschall, 1970; Hammes, 1971; Zelazny, 1973; 1977a; Young, 1974; Bedford, 1976, 1977). Consequent to its introduction, the virus had rapidly spread and established, thereby effectively containing the population of *O. rhinoceros* below the economic injury level (Hammes and Monsarrat, 1974; Zelazny, 1977b; Bedford, 1980). It is claimed to be one of the most successful microbial control agents employed against an insect pest (Caltagirone, 1981). Natural occurrence of the virus in the beetle population has been reported from the philippines and Indonesia (Zelazny, 1977b). This publication reports the occurrence of *Oryctes* baculovirus in nature among the *O. rhinoceros* beetle population in Kerala.

MATERIALS AND METHODS

Collection

O. rhinoceros adults were collected from infested crowns of 5-15 year old coconut palms in farmers' gardens located at Kayangulam, Kappil, Thamarakulam, Bharanicavu, Kattanam, Muttom, Sasthamkotta, Karunagappally, Thazhava, Thodiyur and Sooranad of Alleppey and Quilon districts and Kasaragod in Cannanore district. The beetles were dissected immediately after collection.

Diagnosis

Baculovirus disease in the beetles was diagnosed by *(i) visual examination of the midgut and its contents:* The

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procedure described by Zelazny (1978) was followed; *(ii) Giemsa stained smear of midgut contents:* Air dried smears of midgut contents, aspirated with 1 ml tuberculin syringe, were fixed in methanol for 2–5 min, stained in Giemsa's stain for 1 hr (Lillie, 1965) and finally rinsed in distilled water; *(iii) Immuno-osmophoresis (IOP):* This technique as described by John (1965) was employed with slight modifications, to detect *Oryctes* baculovirus in the midgut aspirate of field collected beetles. Antisera to *Oryctes* baculovirus were obtained from DSIR, New Zealand and also through NCBC, Bangalore, India. Phosphate buffer, 0.045M, pH 7.4, was used both in the agar gel (0.85%) and the buffer tanks. A current of 12–15 mA and a voltage of 10–15 v/cm of agar gel were used. The agar slide containing the midgut aspirates and antiserum in appropriate wells, was placed over a small petridish (7 cm diameter) containing crushed ice to cool the agar during electrophoresis. *(iv) Bioassay test: Inoculation of O. rhinoceros grubs and beetles:* The entire gut of the field collected diseased beetle was excised and homogenised in minimum volume of phosphate buffer, 10^{-3}M, pH 8.5, containing antibiotics (Streptomycin 0.5 g/l, Aureomycin 0.3 g/l; Chloramphenicol 0.3 g/l) and clarified by centrifugation. The homogenate/ midgut aspirate was used to infect healthy third instar *Oryctes* grubs by force feeding. The grubs were slightly anaesthetized with ether and 0.2 ml of the homogenate was administered into the foregut using a polyethylene cannula (No. 47, 1 mm external dia.), attached to a tuberculin syringe. The inoculated grubs (five per container) were maintained in moist autoclaved cattle dung–saw dust mixture (2: 1 w/w) and observed for five weeks for the appearance of symptoms characteristic of the virus disease (Huger, 1966). Appropriate controls were maintained by administering only buffer. At the onset of symptoms of disease the morbid grubs were dissected and the midgut fluid further subjected to IOP for the detection of baculovirus. If one inoculated grub in a test group exhibited typical disease symptoms, it was considered to be bioassay positive.

Freshly emerged adult beetles were partially submerged in the gut homogenate suspension for 30 min, later fed on banana slices smeared with the homogenate. The beetles were dissected after 15 days and the virus disease diagnosed by smear test and IOP. *(v) Electron microscopy (EM):* Beetle midgut slices (1 mm thick) were fixed in 2% glutaraldehyde in phosphate buffer (0.2 M, pH 7.2) for 1 hr at 4°C, washed in buffer thrice and subsequently transported in the same buffer to the Institute of Virology, Oxford, for osmium fixation and staining, ultrathin sectioning and EM examination.

RESULTS AND DISCUSSION

Visual symptoms

The midguts of diseased beetles were white, swollen, filled with a mucoid milky fluid containing flakes of cellular debris, apparently sloughed off from the midgut as a consequence of viral multiplication. These visual lead symptoms were consistently observed in advanced stages of disease and were

partially present or totally absent in early stages of infection. In contrast, the midguts of healthy beetles were very thin, brown and contained very little clear brownish fluid. Another associated feature of the infected midgut was the absence of melanisation at the site of injury on puncturing the midgut whereas healthy midgut showed instant melanisation.

Smear of midgut contents

Giemsa stained smears of diseased midgut contained large clumps of cells with purple stained hypertrophied nuclei and sparse blue cytoplasm (Fig. 1). The infected nucleus strikingly differed from the normal in its hypertrophied appearance (18–28 μm) and contained a homogeneously stained deep pink circular band along the periphery of the nucleus and a central core of still darkly stained granular network (Fig. 2), suggesting derangement of host nucleus and formation of viroplasm. Very often a vacuole like spherical unstained area was seen eccentrically positioned within the nucleus and it was constant characteristic feature of the infected nucleus. Smears of cells in the advanced stages of infection contained only masses of hypertrophied nuclei and very less cytoplasmic material.

The number of free cells in the smears of healthy midgut was significantly less and these cells had a well defined nucleus surrounded by a regular blue cytoplasm (Fig. 3). The healthy nucleus was considerably smaller in size (7.5–12.5 μm) and the purple stained chromatin network imparted a speckled appearance to the nucleus (Zelazny,

FIGS. 1—3. BACULOVIRUS INFECTED AND NORMAL MIDGUT CELLS

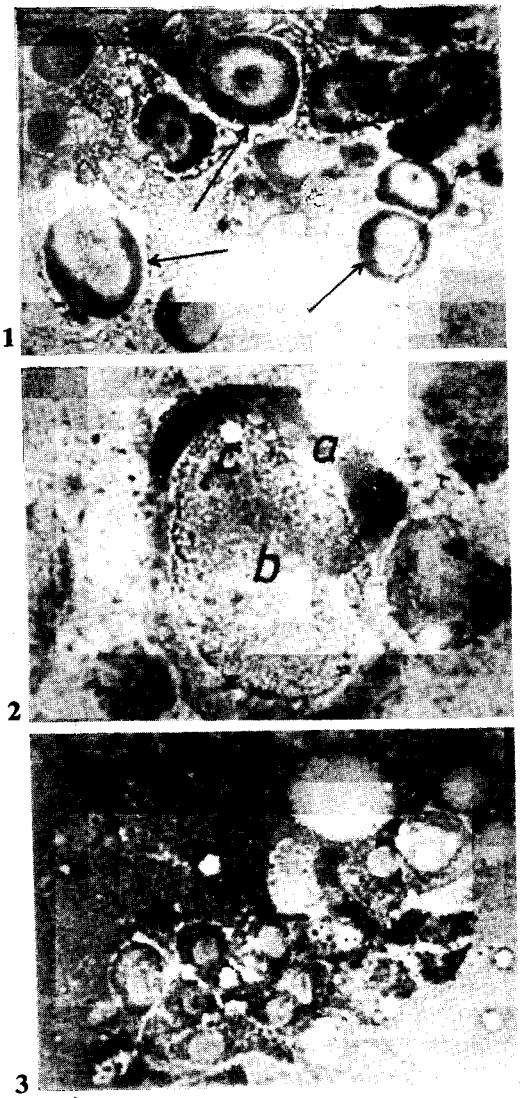


Fig. 1. Infected midgut cells with hypertrophied nuclei in typical 'ring' stages (arrows). x 245.

Fig. 2. Single infected nucleus showing (a) homogeneously stained periphery, (b) granular core and (c) spherical unstained area. x 1,225.

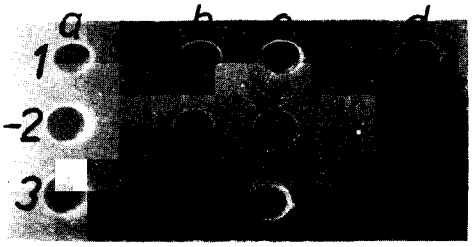
Fig. 3. Healthy midgut smear showing a clump of normal cells. x 245.

1978; Gorick, 1980). Apparently healthy looking cells occurred along with infected cells in various proportions reflecting the stages of the disease.

Immuno-osmophoresis

This technique was used as a rapid method for the detection of baculovirus in the midgut aspirates of diseased beetles. The specific precipitation line formed between the antigen and antiserum wells could be seen after 70 min. of electrophoresis (Fig. 4). Normal rabbit serum served as control. Rarely the gut aspirate of healthy beetles gave rise to a thick yellowish brown precipitation band against the antiserum as well as normal rabbit serum. This non-specific precipitation could be easily distinguished from the specific serological reaction.

FIG. 4. IMMUNO-OSMOPHORESIS OF MIDGUT ASPIRATES



Antigen wells (1a, 2a, 3a) with midgut aspirates of infected *Oryctes* grubs; (1c) baculovirus from South Pacific Islands; (2c, 3c) midgut aspirates from diseased beetles; antiserum wells 1b, 2b, 3b, 1d, 2d & 3d.

The aspirates of diseased beetles which did not evoke positive reaction in IOP predominently contained normal looking cells interspersed with very few infected cells. Possibly, in such cases the virus titre could be below the threshold level for a visible precipitation reaction.

The observation that antiserum to *Oryctes* baculovirus prevalent in the South Pacific Islands, cross reacted with the local *Oryctes* baculovirus suggests strong antigenic relationship. It remains to be investigated if the two viruses are antigenically identical or related.

Bioassay

Healthy third instar grubs force fed with midgut aspirate or gut triturate developed characteristic symptoms of baculovirus disease (Huger, 1966). Healthy beetles infected with the viral suspension contracted the disease after two weeks as evidenced by the diagnostic tests.

The reliability of visual symptoms, smear test and IOP in the diagnosis of baculovirus disease in 96 beetles are given in Table I. The bioassay is not included for comparison, eventhough it was conducted simultaneously with the other tests for 96 beetles, due to mortality of grubs without exhibiting characteristic disease symptoms within a week after inoculation, in about 50% of the test groups and high mortality in the control groups. Out of 55 beetles inferred as diseased (Table I), 58.2% of midguts (32/55) showed typical visual signs of infection and the remaining ones showed partial symptoms, designated as doubtful. Nevertheless, with the inherent shortcomings, the visual symptoms could be considered to be a quick indicator of baculovirus infection

Table I. *Comparison of diagnostic methods for baculovirus disease in* Oryctes rhinoceros *beetles*

Visual	Smear	IOP	Frequency of occurrence (Beetles)	Inference
+	+	+	32	Diseased
D	+	+	17	
D	+	—	3	
—	+	—	3	
D	—	—	8	Healthy
—	—	—	33	
			96	

D – Doubtful symptoms

(Zelazny, 1978). The smear test had been the most consistent of all the three in diagnosing the disease, for all the beetles inferred as diseased gave positive smear test and there was no instance when the smear test was negative and the other two positive. IOP could detect baculovirus in 49 out of 55 diseased beetles (89.1%).

Bioassay had confirmed the disease in all the twenty five beetles inferred as diseased by the other diagnostic tests (Table II). In addition, baculovirus disease in bioassay positive grubs was further confirmed by IOP using the midgut fluid. Three 'healthy' beetles showed positive response in bioassay tests (Table II). This could be due to cross contamination from the adjoining diseased groups of grubs, since it was very unlikely to have missed the disease symptoms in the smear test. Considerable precautions to prevent cross contamination between test groups during the observation period of five weeks, have to be taken and in addition large number of laboratory bred, disease free grubs are needed. The cumbersome nature of the bioassay test underscores its ready utility as a rapid diagnostic test. In view of the high degree of reliability and rapidity the smear test and IOP are proved to be ideal for the screening of a large number of beetles for assessing the natural incidence of disease.

Electron microscopy

Electron micrographs revealed massive infection of the midgut cells with bacilliform virus particles within the nucleus (Fig. 5). The rods measure 215–260 nm in length and 77–108 nm in breadth. The *Oryctes* baculovirus from the South Pacific Islands has been reported to be in the size range of 200 ± 10 nm × 86 ± 15 nm. Each nucleocapsid is enveloped with a distinct membrane.

The locations surveyed have been clustered into four groups based on distances between them. The first three

FIG. 5. INFECTED NUCLEUS FILLED WITH BACULOVIRUS PARTICLES
x 25,000. BAR = 500 nm

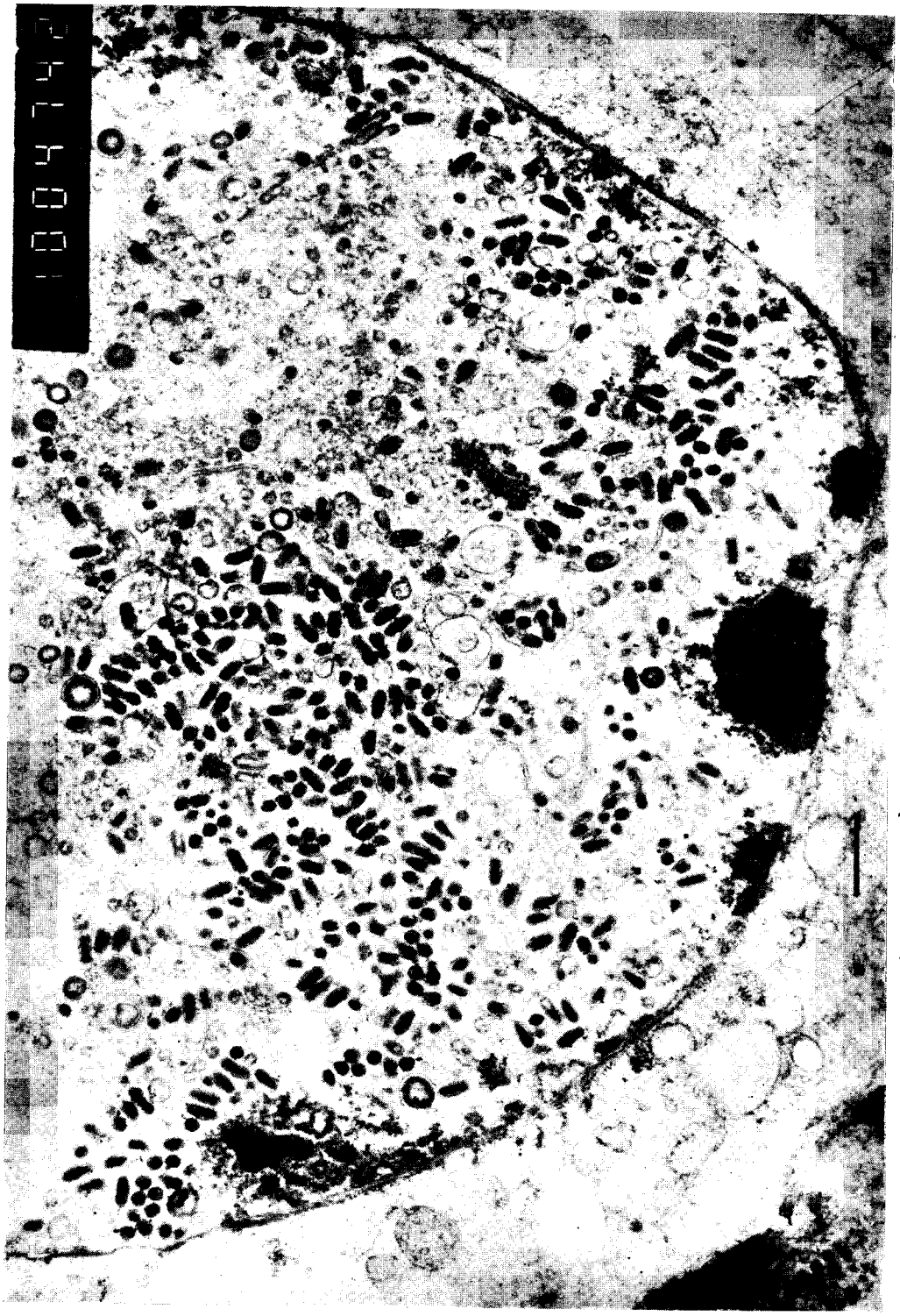


Table II. *Comparison of bioassay with other diagnostic methods*

Consolidated result of visual symptoms, smear test and IOP.	Bioassay	Frequency of occurrence (beetles)	Inference
+	+	25	Diseased
—	+	3	Healthy
+	—	None	
—	—	17	

groups fall within an area, 30 km radius of the Central Plantation Crops Research Institute, Regional Station, Kayangulam. Kasaragod is 350 km north of the Station along the west coast of peninsular India. The percentage disease incidence ranges from 40.9–75.0 with mean of 54.2. The number of beetles examined from Kasaragod area may be too low to reflect the true natural disease incidence, nevertheless, it indicates the presence of the virus. Another factor which is likely to inflate the percentage natural disease incidence is the time for which the beetles are confined together after collection, due to cross infection.

The natural incidence of baculovirus disease among *Oryctes* beetles in the Philippines, Borneo and Central Sumatra (Zelazny, 1977b) was 29% and only 4% in the oil palm plantations of North Sumatra. Comparing the natural incidence of disease, it is relatively high in Kerala and its impact on the intensity of infestation and crop damage by the beetle is to be investigated to assess the effectiveness of this virus as a microbial control agent.

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