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Infection of the coconut palm beetle, *Xylotrupes gideon* (Coleoptera: Scarabaeidae), by a nonoccluded baculovirus

Infektion des Kokosnußpalmenkäfers, *Xylotrupes gideon,* (Coleoptera: Scarabaeidae) durch ein nicht umhülltes Baculovirus

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Summary

Xylotrupes gideon L., a pest of the coconut palm (*Cocos nucifera* L.), was naturally infected by a nonoccluded baculovirus. The virions are rod-shaped, enveloped and have rounded ends with dimensions of 154–185 × 62–69 nm (average 173 × 66 nm) in thin sections. The rod-shaped nucleocapsids with square ends measured 100–112 × 23–29 nm (average 108 × 27 nm). From its morphology and cytopathic effects, the virus very much resembled the nonoccluded baculovirus of the coconut rhinoceros beetle, *Oryctes rhinoceros,* an effective biocontrol agent against this pest. Virus isolated from naturally infected *X. gideon* and *O. rhinoceros* beetles cross-infected both hosts.

Key words: *Oryctes* baculovirus; nonoccluded; cross-infection; *Xylotrupes gideon;* pest of coconut; *Cocos nucifera; Oryctes rhinoceros*

Zusammenfassung

Xylotrupes gideon, ein Schädling an der Kokosnußpalme (*Cocos nucifera* L.), war natürlich infiziert mit einem nicht umhüllten Baculovirus. Die Virionen sind stäbchenförmig, von einer Hülle umgeben und die Enden sind abgerundet. In Dünnschnitten haben sie Dimensionen von 153–185 × 62–69 nm (Durchschnitt 173 × 66 nm). Die stabförmigen Nukleocapside haben eckige Enden und messen 100–122 × 23–29 nm (Durchschnitt 108 × 27 nm). Aufgrund seiner Morphologie und zellschädigenden Effekte ähnelt das Virus sehr dem nicht umhüllten Baculovirus des Kokosnuß-Rhinozeroskäfers, *Oryctes rhinoceros,* einem effektivem Agens zur biologischen Bekämpfung dieses Schädlings. Virus, das aus natürlich infizierten *X. gideon*- und *O. rhinoceros*-Käfern isoliert worden war, führte zu Kreuzinfektionen beide Wirte.

Stichwörter: *Oryctes*-Baculovirus; nicht umhüllt; Kreuzinfektion; *Xylotrupes gideon;* Schädling an Kokosnußpalmen; *Cocos nucifera; Oryctes rhinoceros*

1 Introduction

Xylotrupes gideon L. (Coleoptera: Scarabaeidae) is a pest of the coconut palm (*Cocos nucifera* L.) and coexists with the coconut rhinoceros beetle (*Oryctes rhinoceros* L.) (Menon and Pandalai 1960; Bedford 1975). The beetles feed on the under surface of the young petioles and gnaw the freshly opened

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inflorescences resulting in the breakage of the distal portions of the fronds and reduction in nut production (MENON and PANDALAI 1960; BEDFORD 1980). Though the pest has the potential to cause more economic damage to the crop than the coconut rhinoceros beetle (MENON and PANDALAI 1960), low populations and infrequent infestations on palms classify this beetle only as a minor pest that commonly needs no specific control measures. As many as 16 geographic subspecies of *X. gideon* occur from India eastwards through south-east Asia and extends as far as Papua New Guinea, Queensland, the Solomon Islands and the New Hebrides (BEDFORD 1975, 1980). In contrast, *O. rhinoceros* is a devastating pest of coconut palms and young oil palms. In 1963, a virus disease of this beetle was detected during extensive field surveys in Malaysia (HUGER 1966). The viral pathogen is a nonoccluded baculovirus belonging to subgroup C of the genus *Baculovirus* within the family Baculoviridae. Via the peroral route, both larval and adult stages are highly susceptible to the virus, referred to here as *Oryctes* nonoccluded baculovirus (*Or*NOBV). The infection spreads to nearly all organs and tissues of larvae and adults, yet the nuclei of the midgut and fat body cells are the principal sites of virus reproduction (HUGER 1966, 1972/73; MONSARRAT et al. 1973; PAYNE 1974). A series of related dynastine agricultural pests also proved to be more or less susceptible to the *Or*NOBV, e.g., *Oryctes nasicornis, Oryctes monoceros, Oryctes boas* (HUGER 1966; JULIA and MARIAU 1976; PURRINI 1989). *Scapanes australis grossipunctatus* (BEDFORD 1973), and *Papuana uninodis* (ZELAZNY et al. 1988). Moreover, the *Or*NOBV was shown to multiply in primary *O. rhinoceros* cardiac cell cultures (QUIOT et al. 1973) and in culture cell lines of *Spodoptera frugiperda* and *Aedes albopictus* (KELLY 1976). In extended safety tests with warm-blooded animals and vertebrate cell lines, no sign of pathogenic effects of *Or*NOBV were noticed (ANONYMOUS 1973; GOURREAU et al. 1982), nor is there any evidence that the *Or*NOBV is infective for natural enemies of its homologous hosts. Due to unique pathological process in the midgut of virus-infected adults (HUGER 1972/73), the *Or*NOBV was successfully introduced into *O. rhinoceros* populations of South Pacific and other countries where it became persistently established, thus suppressing this severe pest already for decades (BEDFORD 1981; HUGER and KRIEG 1991).

During a field survey on the incidence of baculovirus infections in *O. rhinoceros* populations of different locations in southern India, *Xylotrupes gideon* beetles were collected from Dakshina Kannada district of Karnataka. Diagnostic studies revealed infections by a nonoccluded baculovirus in the adults of *X. gideon.* The histopathology, morphological characters of the virus and cross-infections of *O. rhinoceros* larvae in the laboratory are reported in this communication.

2 Materials and methods

Behavioural characters of diseased (field-collected) *X. gideon* beetles and external appearence of the infected gut were recorded. Gut aspirate and midgut tissues were smeared and stained with Giemsa solution (LILLIE 1965) for study of histopathological and cytopathic changes (HUGER 1966, 1972/73; MOHAN et al. 1983) under a light microscope.

Midgut tissues (1 × 1 mm) were fixed in 2.5 % glutaraldehyde (0.1 mol/l phosphate buffer, pH 7.4), post-fixed with 1.0 % osmium tetroxide (prepared in above buffer), stained in 0.5 % aqueous uranyl acetate, dehydrated in graded alcohol-acetone series and embeded in Spurr-resin. Ultrathin (60 nm) sections stained with 2 % uranyl acetate and Reynold's lead citrate were observed under a Carl Zeiss 109 transmission electron microscope (TEM) operating at 80 KV. For comparison, *Or*NOBV-infected midgut tissues of adults of *O. rhinoceros* were similarly processed and observed under the TEM. Midgut tissues of *O. rhinoceros* larvae cross-infected by the virus of *X. gideon* were also processed simultaneously to check infectivity and to compare the characters of the virus with those of the natural host.

Acutely infected midguts (whitish, without food) of adults of *X. gideon* were dissected out, triturated in chilled 0.05 mol/l phosphate buffer, pH 8.5 (gut (fr. wt.): buffer (vol.): 1:9) containing 100 µg/ml penicillin and streptomycin each. The triturate was filtered through several layers of cheesecloth and centrifuged at 10 000 ×g for 10 min at 3° C. The supernatant was passed through a 0.22 µm membrane filter. The filtrate was serially diluted with phosphate buffer (0.05 mol/l, pH 8.5) up to 10^{-5} gut·extract equivalent. Laboratory-reared first, second and third instar larvae (20 each) of *O. rhinoceros* were force-fed separately with 0.25 ml of each dose of virus inoculum. Control larvae were

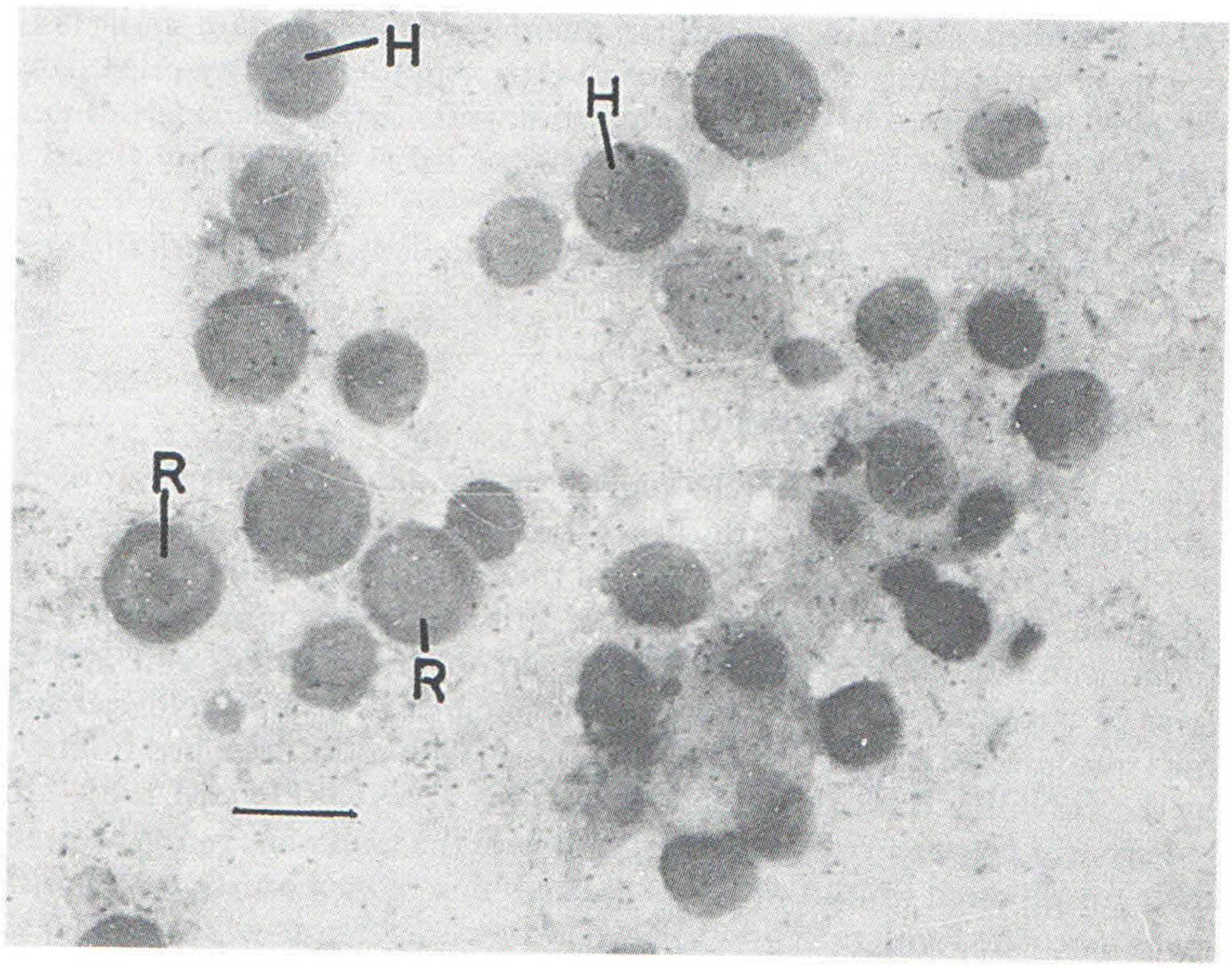

Fig. 1. Virus-infected midgut cells of an adult of *Xylotrupes gideon* showing hypertrophied nuclei (H) with peripheral ring-zone (R). Bar = 25 µm.
Abb. 1. Virusinfizierte Mitteldarmzellen aus einem Imago von *X. gideon,* welche hypertrophierte Kerne (H) mit peripheren Ringzonen (R) zeigen. Strich = 26 µm.

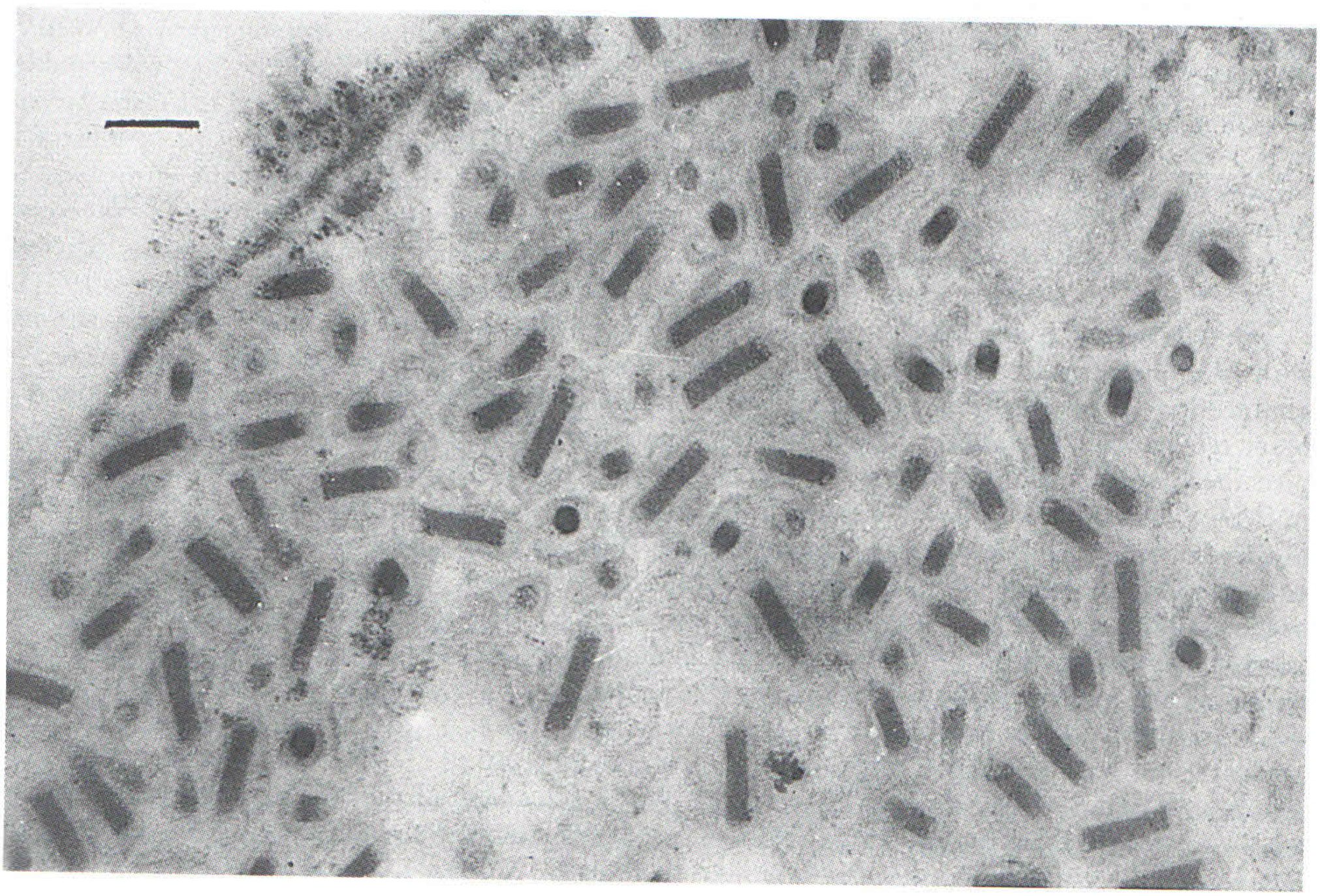

Fig. 2. A midgut nucleus of an adult of *Xylotrupes gideon* at an advanced stage of infection filled with virions and very few spherical envelopes. Bar = 100 nm.
Abb. 2. Kern einer Mitteldarmzelle aus einem Imago von *X. gideon* in einem fortgeschrittenen Stadium der Infektion, angefüllt mit Virionen und sehr wenigen kugelförmigen Hüllen. Strich = 100 nm.

fed with buffer. The larvae of same treatment were maintained in sterile (147 kPa, 1 h, three consecutive days) cattle dung. Appearance of disease symptoms was recorded daily. Morlibund larvae were dissected, appearance of gut tissues and histopathological characters of gut and fat tissues were also checked from Giemsa-stained smears. Median lethal dose (LD_{50}) (Reed and Muench 1938) and median lethal time (LT_{50}) (Litchfield 1949) were calculated from five replicated experiments. For comparative studies, *O. rhinoceros* larvae were similarly treated with the *Or*NOBV.

3 Results and discussion

Infected beetles of *X. gideon* were sluggish; their midgut was dilated, turned milky white and necrotic while the epithelial layer was thickened and cells sloughed-off in the gut lumen. The infected nuclei were hypertrophied up to 2–2.5 times of their normal size and displayed a peripheral ring-zone at the advanced stage of infection (Fig. 1). At an early stage of cell infection, vacuolation of the cytoplasm was prominent, but later the nuclei occupied almost the whole cell and were surrounded by only a thin layer of cytoplasm. Above mentioned morphological, histopathological and cytopathic characters of the virus-infected midgut of *X. gideon* conform with those described from *O. rhinoceros* adults infected by the *Or*NOBV (Huger 1972/73; Mohan et al. 1983; Purrini 1989; Huger and Krieg 1991).

Larvae of *O. rhinoceros* cross-inoculated with the virus of *X. gideon* became translucent and sluggish, their gut appeared whitish without food and their rectum occasionally prolapsed comparable to specimens inoculated with the *Or*NOBV. Giemsa-stained smears of the midgut showed the same histological and cytopathic changes as known from larvae and beetles naturally or artificially infected by the homologous *Or*NOBV (Mohan et al. 1983). TEM studies of midgut nuclei of *O. rhinoceros* infected by the *X. gideon*-virus revealed similar virus structures and symptoms like those infected by *Or*NOBV (Mohan et al. 1983). The three larval instars of *O. rhinoceros* responded similarly (i.e., comparable LD_{50} and LT_{50} values) to the viruses of both *X. gideon* and *O. rhinoceros* (Table 1). First instar larvae were more susceptible to both these viruses with LD_{50} values of 2.11×10^{-3} and 1.13×10^{-3} gut extract equivalent virus inoculum/larva, and LT_{50} values of 10.32 d and 10.45 d against the viruses of *X. gideon* and *O. rhinoceros,* respectively (Table 1). Altogether, the results obtained with *O. rhinoceros* larvae infected by the *X. gideon*-virus are comparable to those described from larval specimens infected by their homologous virus (Payne 1974; Mohan et al. 1983; Purrini 1989; Huger and Krieg 1991).

Thin sections of adults showed that at an early stage of infection an amorphous virogenic stroma with small electron dense bodies is formed in the nuclei. Subsequently, rod-shaped virions were assembled in vesicles around the peripheral ring-zone and large numbers of spherical vesicles (unit membrane type) encircling a darker central core (different stages of morphogenesis of nucleocapsids) occupied the central portion of the nuclei. Occasionally, a large electron dense, ring-like disintegrating nucleolus was also noticed in the nucleus at the initial stage of infection. Heavily infected nuclei were filled with rod-shaped virions and very few vesicles (Fig. 2). The virions were always loosely covered with a double membrane envelope and had rounded ends. Rarely naked nucleocapsids but no virus occlusion bodies could be noticed in infected cells (Fig. 2). The virions never budded but were released by rupturing of the nuclear membrane. Sometimes, groups of virions but no virogenic centres were observed in the cytoplasm. The rodshaped enveloped virions measured 154–185 nm in length (n = 112) and 62–69 nm in width (n = 86) with an average of 173 × 66 nm (Fig. 2, Table 2). The nucleocapsids were rodshaped with square ends measuring 100–112 × 23–29 nm (average 108 × 27 nm; n = 94 and 84, respectively) (Fig. 2, Table 2). The diameter of the spherical vesicles was from 45–94 nm (average 68 nm, n = 45). Shape and size of the virions in the nuclei of *O. rhinoceros* cross-infected by the virus of *X. gideon* were similar to those of *X. gideon.* The same is true for virions, nucleocapsids and spherical vesicles of simultaneously processed midgut tissues of *Or*NOBV-infected *O. rhinoceros* beetles (see Table 2). Morphology and size of the virions, nucleocapsids and spherical vesicles as well as cross-infectivity to *O. rhinoceros* and associated cytopathic characters (Fig. 1, 2; Table 2) indicate that the virus infecting *X. gideon* is a nonoccluded baculovirus belonging to Baculoviridae: Subgroup C and is most probably identical to *Or*NOBV (Matthews 1982; Bilimoria 1986). However, as seen from Table 2, the size of the virions, nucleocapsids and spherical vesicles of *Or*NOBV of *X. gideon* and *O. rhinoceros* measured smaller in our investigation than those of Malaysia (Huger

Table 1. Median lethal dose (LD_{50}) and lethal time (LT_{50}) of first, second and third instar larvae of *Oryctes rhinoceros* infected by the baculoviruses of *Xylotrupes gideon* and *O. rhinoceros*
Tab. 1. Mittlere letale Dosis (LD_{50}) und letale Zeit (LT_{50}) der ersten, zweiten und dritten Larvenstadien von *O. rhinoceros* infiziert mit dem Baculovirus von *X. gideon* und *O. rhinoceros*

Source of virus	LD_{50} ($\times 10^{-3}$) of instars			LT_{50} (d) of instars		
	First	Second	Third	First	Second	Third
Xylotrupes gideon	2.11±0.39	31.14±5.31	2.66±0.42	10.32±3.11	17.19±4.22	15.62±5.19
Oryctes rhinoceros	1.13±0.24	25.69±3.98	1.48±0.22	10.45±1.41	13.25±5.62	13.67±3.10

Results are the mean of five replications ± standard error.

Table 2. Comparative size (length (1) × width (w), nm) of virions and nucleocapsids of baculoviruses from *Xylotrupes gideon* and *Oryctes rhinoceros* observed from different studies
Tab. 2. Größenvergleiche (Länge (1) × Breite (w), nm) der Virionen und Nukleocapside des Baculovirus von *X. gideon* und *O. rhinoceros*, zusammengetragen aus verschiedenen Untersuchungen

Host	Source/ country	1 × w (nm)		Reference
		Virion	Nucleocapsid	
Xylotrupes gideon	Natural/ India	(154–185) × (62–69)	(100–112) × (23–29)	Present study
Oryctes rhinoceros	Natural/ India	(130–156) × (41–76)	(100–117) × (22–35)	Present study
O. rhinoceros	Natural/ Malaysia	(170–210) × 70	NA	Huger 1966
O. rhinoceros	Laboratory/ UK	220 × 120	180 × 60	Payne 1974
O. rhinoceros	Laboratory/ UK	200 × 100	160 × 50	Payne et al. 1977
O. rhinoceros	Natural/ India	(215–260) × (77–108)	160 × 80[a]	Mohan et al. 1983

[a] Calculated by the present authors from the published photograph.
NA – Not available.

1966) and Kerala, India (Mohan et al. 1983). Probably, differences in the processing schedule might be responsible for such variations which were also observed by other authors with *Or*NOBV (Table 2) (Huger 1966; Payne 1974; Payne et al. 1977) as well as with baculoviruses of the nuclear polyhedrosis (NPV) and the granulosis (GV) type (Ackermann and Smirnoff 1983). It is generally agreed that reliable comparisons of baculoviruses on the basis of dimensions can only be made if the tissues are processed under identical conditions (Matthews 1982; Ackermann and Smirnoff 1983; Bilimoria 1986).

Considering the limitations of only morphological and cross-infection studies, it can be concluded that *X. gideon* is a natural host of *Or*NOBV which might have minimized the pest population and the resultant crop infestation in the locality.

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