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Diagnosis of baculovirus infection in coconut rhinoceros beetles by examination of excreta[1)]

Diagnose der Baculovirus-Infektion von Kokosnuß-Nashornkäfern durch Untersuchung der Exkremente

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Summary

Oryctes rhinoceros beetles, when infected with *Oryctes* baculovirus, excreted gut epithelial cells having hypertrophied nuclei, between the third and ninth day post-inoculation. Detection of such abnormal nuclei in the excreta was used as a method of diagnosis of baculovirus infection in field-collected beetles, needed alive for various experiments. This method eliminates the need for sacrificing the beetles for mid-gut examination, a routine method of diagnosis.

Key words: *Oryctes rhinoceros*; baculovirus infection; excretion; epithelial cells; nuclei, hypertrophied; diagnostic method

Zusammenfassung

Oryctes rhinoceros-Käfer, die mit dem Oryctes-Baculovirus infiziert waren, schieden zwischen dem 3. und 9. Tag nach Inokulation Darmepithelzellen mit hypertrophierten Kernen aus. Das Vorhandensein solcher abnormen Kerne in den Exkrementen wurde als Nachweis für Baculovirus-Infektionen bei im Freiland gesammelten Käfern benutzt, die lebend für verschiedene Experimente benötigt wurden. Bei Einsatz dieser Methode erübrigt sich die Tötung der Käfer zur Untersuchung des Mitteldarmes, wie es sonst routinemäßig zur Diagnose üblich ist.

Stichwörter: *Oryctes rhinoceros*; Baculovirus-Infektion; Exkretion; Epithelzellen; Kerne, hypertrophiert; Diagnosemethode

1 Introduction

Oryctes baculovirus (Huger 1966; Payne 1974; Payne et al. 1977) is documented to be one of the few successful microbial control agents ever used for suppression of insect pests (Caltagirone 1981). Introduction of this entomopathogenic virus from Malaysia into the South Pacific Islands in the sixties had led to a remarkable reduction in the pest population of the coconut rhinoceros beetle, *Oryctes rhinoceros* L., below the economic injury level and was sustained at

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this level for many years (MARSCHALL 1970; HAMMES and MONSARRAT 1974; YOUNG 1974; GORICK 1980). The disease was also found to occur in the natural population of the grubs and adults of *O. rhinoceros* in Kerala, India (ZELAZNY 1981; MOHAN et al. 1983).

A large number of field-collected beetles were needed alive for experiments on various aspects of the baculovirus disease. It was essential to know if the beetles were disease-free or not, without dissecting them, since microscopic examination of the mid-gut epithelial cells is the routinely followed diagnostic method (ZELAZNY 1978). Externally it is not possible to differentiate between healthy and diseased beetles, unlike the grubs where the external disease symptoms are strikingly apparent (HUGER, 1966). The objective of this study was to periodically examine the faecal matter of laboratory-infected and field-captured beetles, for sloughed-off gut epithelial cells with the characteristic abnormal nuclei, an indication of baculovirus infection. This is the first report of this kind.

2 Materials and methods

O. rhinoceros adults used in the experiment were reared in the laboratory and also collected from the crowns of infested palms and the breeding sites of the pest. Initially, the progress of baculovirus disease and the consequent excretion of infected gut cells were studied in laboratory-infected beetles. A batch of ten beetles (one-month-old, laboratory emerged) was infected by allowing them to wade through a baculovirus suspension for 30 min (4 g of diseased grub tissue in 100 ml phosphate buffer $\simeq$ 10 LD_{50} doses for *O. rhinoceros* grubs) containing 2 % dextrose, kept in a shallow container. Subsequently, the beetles were confined together in autoclaved cattle dung, treated with the virus suspension , for 24 h. The following day, each treated beetle was kept individually in a plastic container with 5 ml of phosphate buffered saline (0.01 mol/l, pH 7.0; NaCl 0.85 %), just enough to be in level with the distal end of abdomen of the beetle. The buffer contained antibiotics (Streptomycin, 250; Penicillin, 200; oxytetracycline, 100 mg/l) to prevent any possible bacterial action on the excreted gut cells. The faecal matter was collected and centrifuged at 500 rpm for 10 min. The supernatant was discarded and the sediment was resuspended in 0.2 ml buffer by gentle pipetting. A smear was made with a drop of the resuspended sediment, air dried, fixed in methanol for 3 min and stained with Giemsa for 75 min (LILLIE 1965) and examined under oil immersion. Three replications of the experiment were conducted. Field-captured beetles, whose diesease status was to be determined, were confined individually and their excreta examined on alternate days.

The faecal examination was continued for 3 weeks after which the beetles were dissected for examining the mid-gut cells, an established method of confirming the disease.

3 Results and discussion

Fig. 1 represents the consolidated results of three replications conducted to follow the time course of the disease in laboratory-infected beetles. The criterion for inferring a beetle as diseased was the observation of cells with abnormal nuclei in the faecal matter. The gross appearance of infected and normal gut epithelial cells in excreta matched the description of baculovirus-infected mid-gut cells in *Oryctes* beetles (ZELAZNY 1978; MOHAN et al. 1983). Certain additional features were also noted. Excreted infected cells were mostly single and less frequently in clumps of gut tissue sloughed-off due to viral infection of the gut epithelium. The isolated cells had a highly disintegrated cytoplasm, but the most prominent feature was the hypertrophied ring staged nucleus (11.40–19.95 μm) taking dark purple hue (Fig. 2). Frequently, isolated nuclei in ring stages could also be observed. Prior to the appearance of infected cells, the excreta contained a lot of cellular debris till the sixth day post-infection. This was a consistent feature.

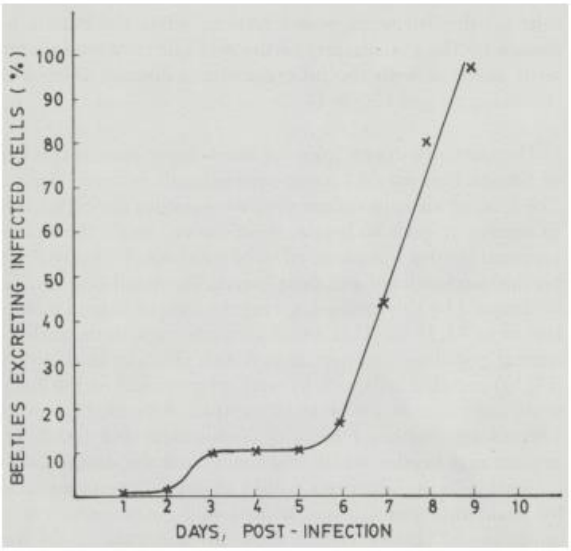

Fig. 1. Time course of disease in laboratory-infected *Oryctes rhinoceros* beetles.
Abb. 1. Zeitlicher Verlauf der Krankheit in künstlich infizierten *Oryctes rhinoceros*-Käfern.

The excreta of healthy beetles (control) contained neither cellular debris nor whole cells on the majority of days. At times, isolated cells were found having considerably smaller (6.5–11.0 μm) but well defined nuclei with chromatin network imparting a speckled appearance.

Infected cells were found in the excreta from the 3rd day post-infection in 10 % of laboratory-infected beetles. The incidence sharply rose on the 7th day (43.33 %) and reached the maximum on the 9th day (96.66 %). All the infected beetles continued excreting infected

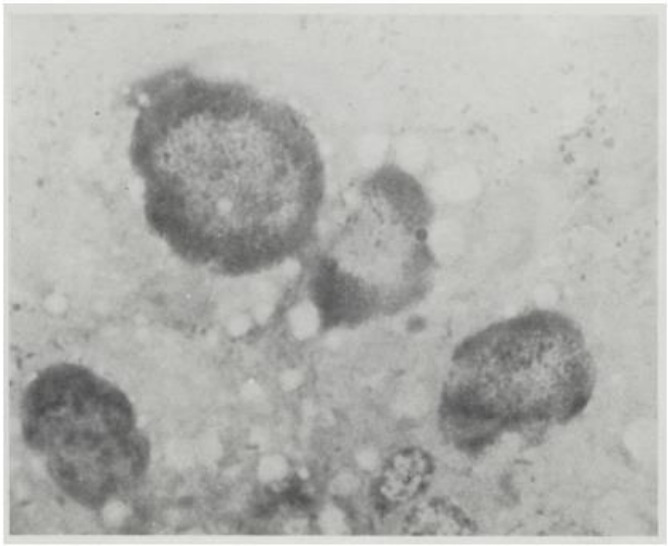

Fig. 2. *Oryctes* baculovirus-infected gut nuclei in the excreta of diseased beetle, showing characteristic 'ring' stage (× 650).
Abb. 2. Mit dem *Oryctes*-Baculovirus infizierte Kerne von Darmepithelzellen mit dem charakteristischen 'Ring'-Stadium in Exkrementen von kranken Käfern (× 650).

cells till the 3rd week post-infection, when the beetles were dissected for confirming the disease by the examination of the mid-gut. It would be interesting to study the quantity of virus excreted with the progress of the disease, using a sensitive assay method as ELISA (LONGWORTH and CAREY 1980).

The inference drawn from the above experiment is that beetles infected with 10 LD_{50} dosage of baculovirus would excrete infected cells between the 3rd and the 9th day post-infection. The level of virus inoculum present in nature during transmission of the disesase from beetle to beetle, or grub to beetle, is unknown, hence the time course of infection in such field-captured beetles was expected to be different. To study this aspect, a total of 66 field-collected beetles was confined and their excreta examined (Fig. 3). Thirty four beetles were inferred to be diseased by this method, giving the level of natural incidence in the fields around Kayangulam to be 51.51 %. This was in conformity with the earlier report of 54.2 % incidence in the natural population of beetles in Kerala (MOHAN et al. 1983). About 38 % of infected beetles (Fig. 3) excreted cells with hypertrophied nuclei on the 2nd day of confinement, reflecting the acute infection at the time of capture. Remarkably, nearly 73 % of the beetles excreted infected cells within 1 week of confinement and 100 % of them within the 12th day. This implies that beetles which had contracted the disesase on the day of capture, would show infected cells in the excreta within 13 days. Disease was confirmed in the group of 34 beetles by examining the Giemsa stained mid-gut smears. On the contrary, the excreta of the remaining 32 beetles did not show any abnormal nuclei containing cells even up to 20 days. These were confirmed to be normal beetles on further mid-gut examination as well.

Based on these results, a criterion for diagnosing the baculovirus disesase in field-captured beetles was fixed. If no infected cells could be observed in the excreta up to 2 weeks after capture, then they were inferred to be disesase-free. This method of diagnosis has been very useful in experiments in which the disesase status of the field-captured beetles was to be known before planning.

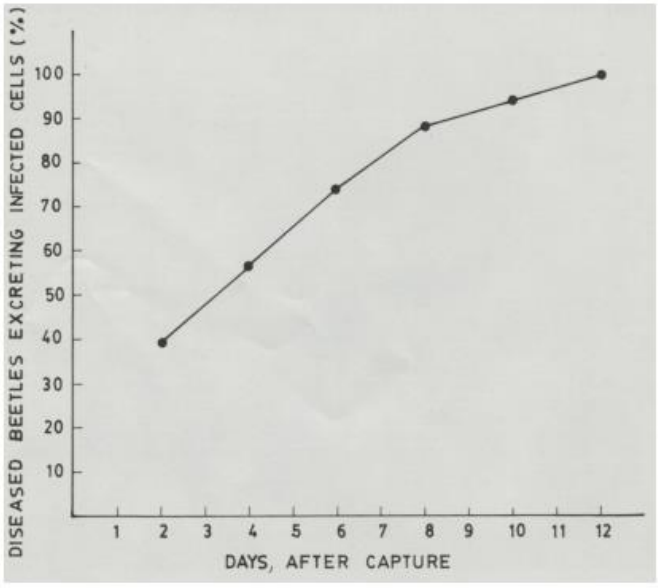

Fig. 3. Time course of disease in field-collected *Oryctes rhinoceros* beetles.
Abb. 3. Zeitlicher Verlauf der Krankheit bei im Freiland gesammelten *Oryctes rhinoceros*-Käfern.

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