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Release and establishment of the baculovirus disease of *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) in Papua New Guinea

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Abstract

Oryctes rhinoceros (L.) is a major pest of coconut palms in Papua New Guinea but has a limited distribution within the country. Pre-release monitoring showed that no baculovirus disease was present in the country prior to its importation. A consignment of *O. rhinoceros* larvae infected with a baculovirus disease was imported into Papua New Guinea from Western Samoa in 1977. The virus proved highly infectious to both larvae and adults of the local population of *O. rhinoceros*. During 1978 and 1979, adults were perorally infected with the baculovirus and released at nine sites on Manus Island, four sites on New Ireland and twelve sites on the Gazelle Peninsula of East New Britain. The infected adults were active disseminators of the virus into the field population, and the virus became established at nearly all the release sites. The shortest time between virus release and recapture of newly infected adults from a release site was eight weeks. Three different examples indicated the virus spread at approximately 1 km/month. During a fourteen-week period in the early establishment phase, significantly more females than males were collected in traps but a significantly higher percentage of the males was infected.

Introduction

Distribution

The rhinoceros beetle, *Oryctes rhinoceros* (L.), is a pest of coconut palms (*Cocos nucifera*) in Papua New Guinea. It has a limited distribution within the country, being found only on Manus Island, New Ireland and the Gazelle Peninsula of East New Britain (Fig. 1). *O. rhinoceros* is not indigenous to Papua New Guinea, but was first introduced during World War II to New Britain (in about 1942) and later arrived on New Ireland (about 1952), Pak Island (1960) (Catley, 1969) and Manus Island (1970) (Bedford, 1976*a*). *O rhinoceros* has not been recovered from other coconut-growing areas in Papua New Guinea despite the opportunity for spread to occur due to frequent inter-island shipping. The level of beetle damage to palms is generally low to moderate, but palms in certain areas suffer severe attacks.

Damage

In Papua New Guinea, *O. rhinoceros* usually attacks palms more than four years of age (Bedford, 1976*a*). The adult flies at night to a palm and chews into the un-

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opened fronds, which on later opening show characteristic v-shaped cuts. Feeding holes made by this beetle, can then be entered by the black palm weevil, *Rhynchophorus bilineatus* (Montrouzier), which often causes palm death.

The baculovirus of Oryctes

The control of *O. rhinoceros* in other countries improved dramatically following the discovery of a new type of insect virus in this species in Malaysia in 1963 (Huger, 1966). Huger named this virus *Rhabdionvirus oryctes*, but it was later reclassified as belonging to the new *Baculovirus* group (Monsarrat et al., 1973, Payne, 1974). Larvae of *O. rhinoceros* infected with this baculovirus generally exhibit several of the following distinct symptoms; cessation of feeding; diarrhoea; a translucent appearance; and sometimes a prolapsed rectum. The most active sites of virus replication in larvae are the fat-body and mid-gut epithelium (Huger, 1966). Infected adults show severe histological changes in both the epithelium and regenerative crypts of the mid-gut. The

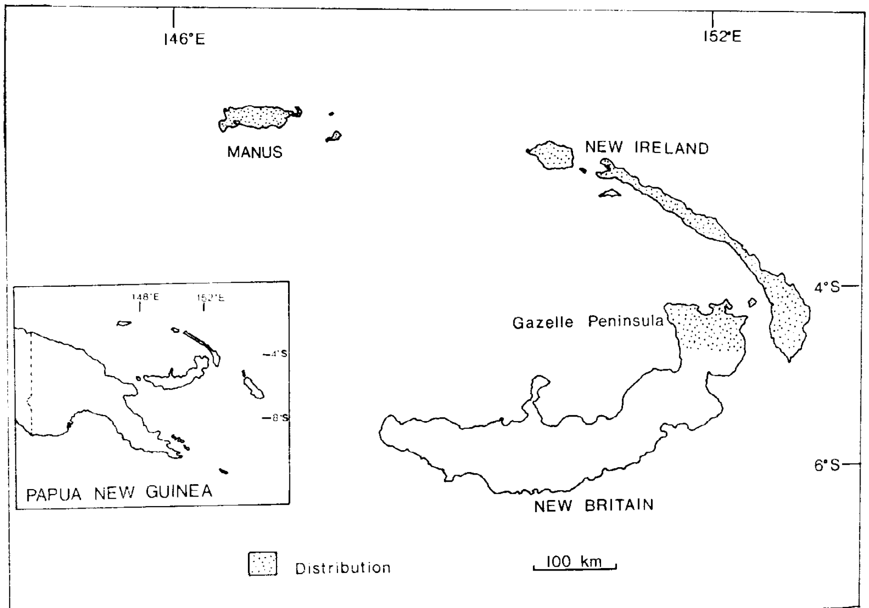

Fig. 1.—The distribution of *Oryctes rhinoceros* in Papua New Guinea.

lumen of the mid-gut becomes filled with large numbers of virus-infected cells. The disease is transmitted when virus-infected nuclei are excreted, and the infected adults pass the virus to healthy adults in the field during mating and through contamination of the breeding sites (Huger, 1973, Zelazny, 1976). Infected adults can pass the infection to larvae when visiting breeding sites, and healthy adults can contract the disease if they visit a breeding site containing infected larvae (Zelazny, 1976). Infected adults have been found to have a shorter life-span and to lay fewer eggs than healthy ones (Zelazny, 1973).

The virus was released in Western Samoa in 1967 (Marschall, 1970), and a marked decrease in palm damage by *O. rhinoceros* was subsequently observed. Further releases were made during 1970 on Wallis Island (Hammes, 1971) and Tonga (Young, 1974), and

in 1971–1974 in Fiji (Bedford, 1976*b*). A frozen culture of the baculovirus of *O. rhinoceros* was first imported into Papua New Guinea from Western Samoa in 1970 and tested against *O. rhinoceros* larvae in the laboratory (Bedford, 1973*a*). The virus proved infectious, but no field releases of the virus were made and none of the original virus stock remained at the start of current work in 1977.

Materials and methods

Pre-release monitoring

Prior to the release of the virus in Papua New Guinea, 126 adult *O. rhinoceros*, 62 from the Gazelle Peninsula of East New Britain, 59 from Manus and 5 from New Ireland, were histologically examined for the presence of virus. More than 2000 larvae were collected in the field prior to the release of virus.

Experimentation with larvae

A consignment of virus-infected *O. rhinoceros* larvae was received from Western Samoa in October 1977 and tested for infectivity. Four of the larvae were finely ground and mixed with 20 litres of autoclaved sawdust to which 50 healthy field-collected *O. rhinoceros* larvae were added. The larvae were frozen after they showed symptoms of virus infection, thus forming a bulk supply of virus. Five replications of virus were made in this manner, each replication using, as inoculum, virus-killed larvae produced in a previous replication.

An alternative method of testing the infectivity of the virus was also carried out. The haemolymph of a moribund larva was removed, and diluted 1 : 1 with boiled distilled water and force-fed to 30 third-instar larvae using a syringe, each larva receiving approximately 1 ml. Twenty control larvae were force-fed with approximately 1 ml boiled distilled water. The larvae were kept in 12 litres of autoclaved rotted sawdust in metal dustbins.

Collection of adult O. rhinoceros *for virus inoculation*

Adult *O. rhinoceros* were either field-collected or laboratory-reared from field-collected third-instar larvae.

Field collections.—Adults were collected in the field by searching palms 5–8 years old or breeding sites. They were kept in autoclaved sawdust in wooden boxes (43 $\times$ 43 $\times$ 26 cm) and fed on sugar-cane until needed.

Laboratory rearing.—Third-instar larvae collected in the field were kept in the laboratory in groups of 100–200 in similar wooden boxes, which had a wire-mesh lid and were half-filled with an autoclaved mixture of rotting sawdust and palm sawdust. When the larvae had entered the prepupal stage, they were placed individually in tins or jars, with sawdust, and left undisturbed until the emergence of the adult. The emergence date of the adult was recorded.

Inoculation techniques used on adults

Swim technique.—To infect adults, two virus-infected larvae were ground up and suspended in 1 litre of distilled water. Adults were immersed in this mixture in batches of five for 2–3 min, after which they were placed in approximately 6 litres of autoclaved sawdust to which the suspension from mixing one ground-up infected larva with 500 ml distilled water had been added. This technique followed that described by Bedford (1976*b*).

Oral inoculum technique.—The swim technique was superseded by the oral-inoculum technique first described by Marschall (in press). The mid-gut was removed from adults 10–12 days after they had been inoculated with virus. The mid-guts were weighed, mashed in a tissue grinder and diluted with sufficient distilled water to make a concentration of 10^{-3} g infected gut per 10 μl. This basic stock was then deep frozen.

At the time of inoculation, the stock was diluted ten times with 10% sugar solution. Each beetle was held on its dorsum, and a 10 μl drop of the virus inoculum, containing approximately 10^{-4} g infected gut, was placed on the mouthparts. The beetles readily ingested the sweetened inoculum and were then ready for field release.

Diagnostic techniques

Two histological methods were used in the diagnosis of virus infection in adults.

Serial sections.—Adult mid-guts were embedded in paraffin wax and sections 5 μm thick cut. The nuclear stain was Mayer's haemalum and the counterstain 0·1% eosin.

Rapid smear technique.—This was the main diagnostic technique used. Small pieces of mid-gut were thoroughly squashed to break up the tissue and release the epithelial nuclei. A thin smear was made, fixed with alcohol and stained with 10% Gurr's Improved R66 Giemsa.

Release of virus-infected adults

All releases of the virus were via infected adults, inoculated perorally. Before release on the Gazelle Peninsula of East New Britain, all inoculated beetles were scratched with a code on the elytra in order to be able to distinguish them from the field population. Inoculated beetles were taken to the release sites, placed on the ground and allowed to crawl away. Up to November 1979, the numbers of infected adults that had been released were 250 at nine sites on Manus, 247 at four sites in New Ireland and 920 at twelve sites on the Gazelle Peninsula.

Post-release monitoring

To determine whether the virus had become established in the field population after releases of inoculated beetles, adults were either collected by hand in the field (Manus and New Ireland) or caught in traps (Manus, New Ireland and Gazelle Peninsula).

Gazelle Peninsula.—At each release site on the Gazelle Peninsula, traps were erected at the time of virus release. The traps followed the design of Bedford (1973*b*) and used ethyl chrysanthemumate (Rhinolure) as attractant (Maddison et al., 1973). Four traps were set up near each release site (except Keravat, which had six traps) and were inspected at approximately ten-day intervals. All beetles found were placed in individual jars. In addition, traps were erected outside the release areas at Watta (5 km from the release site, Tokua) and at Matupit (approximately 7 km by road from Malaguna). To monitor the spread of virus, traps were set up in each direction along the road and at regular distances from the release site at Malabunga for a total distance of 7·5 km. Similarly, traps were erected to a distance of 8·2 km from the release site at Vuvu. After collection, the beetles were kept in individual containers in the laboratory for approximately ten days before they were dissected and examined histologically. For a 14-week period, from 9 July 1979 to 14 October 1979, all beetles collected from traps on the Gazelle Peninsula were sexed during dissection.

Manus and New Ireland.—No regular trapping programme was carried out on either Manus or New Ireland. The release sites were irregularly visited and beetles collected either from traps or by hand from palms.

Palm damage surveys

In an attempt to measure population levels of *O. rhinoceros* by frond damage, survey blocks were set up at five release sites on Manus (Salasea, Salami, Lugos, Nuwok and Momote), three release sites on New Ireland (Koka, Bopire and Kimadan) and eight sites on the Gazelle Peninsula (Tokua, Varzin, Raluana, Malabunga, Vuvu, Matupit, Keravat and Raulawat). Each month, the most recently opened frond on all the 100 marked palms at each survey site was recorded as either undamaged or damaged.

Results

Pre-release monitoring

All 126 adult *O. rhinoceros* examined histologically prior to the release of the virus were healthy. In addition, between the dates of release of the virus at various sites on the Gazelle Peninsula and the times of the first recovery of infected adults, 281 uninfected beetles were caught in traps; 156 of these were from within the virus-release sites and 125 were from other areas. Of more than 2000 larvae collected from the field prior to the release of the virus, none showed any symptoms of virus infection.

Effect of virus on larvae

All *O. rhinoceros* larvae ($N = 435$) inoculated with the baculovirus died within 28 days post inoculation (d.p.i.). After the same period of time, 93% of 70 control larvae were still alive. In four of the five virus replication experiments, no larvae survived longer than 18 d.p.i. and more than 50% of the larvae died between six and ten d.p.i. (Fig. 2). The dead larvae all showed the typical symptoms associated with this viral disease (Huger, 1966).

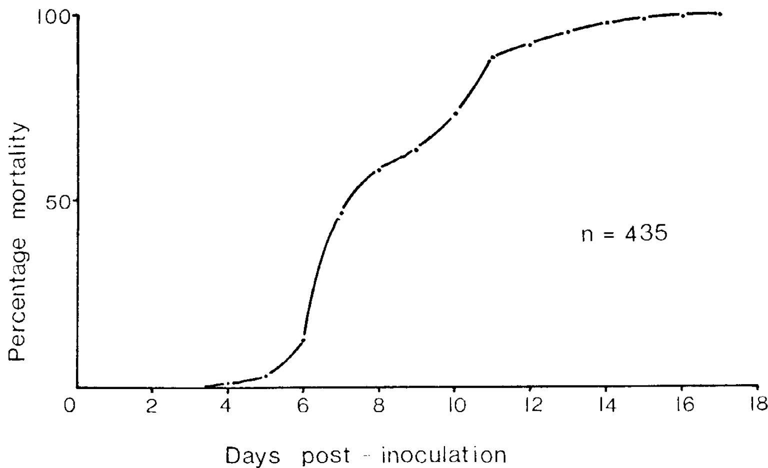

Fig. 2.—The percentage mortality of third-instar larvae of *O. rhinoceros* infected with the baculovirus.

The force-feeding experiment with haemolymph produced similar results, with more than 50% of larvae dying between 6 and 15 d.p.i. Mortality in the controls was 7% at 19 d.p.i.

Effect of virus on adults

Infectivity.—Both the swim technique and oral inoculum technique resulted in infection of adults in laboratory trials. In several small trials a high percentage of inoculated beetles became infected. All controls remained healthy.

Histological effects.—Serial sections of the mid-gut of infected adults showed classic virus symptoms as described by Huger (1973) and Monty (1974). Nuclei in the mid-gut epithelium were densely stained, the regenerative crypts were actively proliferated and at the advanced stages of infections (more than 10 d.p.i.) the lumen became completely filled with a mass of virus-infected cells.

Smears of mid-guts of adults were very distinctive. Healthy nuclei were small and contained patches of purple-stained chromatin, which gave the nuclei a speckled appearance. By contrast, virus-infected nuclei were hypertrophied, and appeared homogeneous and pink, often with ring zones within the nuclei. Smears were the most frequently used means of diagnosing baculovirus infections. During dissection, differences between healthy and virus-infected mid-guts were often observed.

Visual diagnosis of the mid-gut on dissection as either virus-infected or uninfected was almost always the same as the diagnosis based on smear examination. Healthy mid-guts were commonly thin, brown and strong, whilst infected mid-guts were swollen, white and fragile. Both the macrostructure of a virus-infected mid-gut and the appearance of the infected nuclei it contained were similar to that described by Zelazny (1978). Occasionally, infected mid-guts were observed to be almost totally degenerated and contained only brown gritty substance in the lumen.

Electron micrographs of the mid-gut of one adult inoculated using the oral inoculum technique showed heavy virus replication within a nucleus. The virus structure appeared identical to that depicted by Huger (1973), being an enveloped DNA virus without inclusion bodies. Histological examination of infected insect tissues using both the light microscope and electron microscope indicated that the virus was likely to be the same baculovirus used in the control programme in Western Samoa.

The mid-guts of some beetles examined had protozoan infections. The protozoan, probably a gregarine (Huger, pers. comm.), was not identified. Similar protozoan infections of *O. rhinoceros* had been observed by Huger (1968). The presence of protozoa was independent of virus infection and did not affect the diagnosis of virus infection in the mid-gut epithelium.

Release and recovery of the virus

The dates of virus releases at sites in Papua New Guinea, the number of marked infected beetles released and the dates on which unmarked infected beetles released

TABLE I. *Release sites, dates of release and dates of first field recovery of infected adult* O. rhinoceros

Province	Site	Release date	No. of beetles released	Date of first virus recovery from field	Interval (weeks)
Manus	Nuwok	5.i.1978	22	17.viii.1979	*
New Ireland	Koka	4.vii.1979	100	NVR	
Manus	Lugos	14.vii.1978	35	15.ix.1978	9
Manus	Momote	27.viii.1978	40	5.iii.1979	27
Manus	Lombrum	5.ix.1978	17	23.ii.1979 }	22/24
Manus	Lombrum	19.ix.1978	39	23.ii.1979 }	
Manus	Salami	10.xi.1978	27	9.ii.1979	13
Manus	Salasea	24.xi.1978	30	16.viii.1979	38
Manus	Nuwok	24.xi.1978	32	17.viii.1979	38
New Ireland	Bopire	27.xi.1978	40	24.iii.1979	17
New Ireland	Kalili	29.xi.1978	27	14.v.1979	24
New Ireland	Kimadan	30.xi.1978	80	5.vi.1979	27
Manus	M'Bunai	26.ii.1979	8	not sampled	
East New Britain	Vuvu	4.iv.1979	50	28.viii.1979	21
East New Britain	Malabunga	6.iv.1979	50	20.viii.1979	20
East New Britain	Tokua	11.iv.1979	50	7.vi.1979	8
East New Britain	Malaguna	16.v.1979	50	28.viii.1979	15
East New Britain	Raluana	22.vi.1979	100	7.ix.1979	11
East New Britain	Vudal Beach	25.vi.1979	100	27.xi.1979	22
East New Britain	Keravat	26.vi.1979	100	4.ix.1979	10
East New Britain	Raulawat	12.vii.1979	100	27.ix.1979	11
East New Britain	Varzin	31.vii.1979	83	NVR	
East New Britain	Kabaira	14.viii.1979	83	NVR	
East New Britain	Vimy	11.x.1979	61	NVR	
East New Britain	Vunapalading	2.xi.1979	93	NVR	

*Assumed not established.
NVR = no virus recovery to November 1979.

and the date on which unmarked infected beetles were first recovered from the field are shown in Table I. In only the first two of these releases, at Nuwok and Koka, were the beetles inoculated by the swim technique; all other releases used the oral inoculum technique.

The virus was recovered from all sites on Manus that were revisited. No beetles had been collected up to November 1979 from M'Bunai, and no other collections from sites outside the original virus release sites were made on Manus.

The virus was recovered from three sites on New Ireland. Five beetles were collected from Koka during November 1979 and all were histologically negative for virus. At Patlangat, 15 km from Koka, 14 uninfected beetles were collected at the same time.

On the Gazelle Peninsula of East New Britain, virus-infected beetles were recovered from eight of the 12 virus release sites by November 1979. Traps in all areas were visited at about ten-day intervals. Virus recovery was notably rapid at the Tokua site, with the first unmarked infected beetle being collected from a trap eight weeks after the release of marked infected beetles. After a further month, 12 infected and three uninfected beetles were collected from four traps at Tokua, which were then dismantled. At Malaguna, the site of an *O. rhinoceros* outbreak in 1979 due to extensive felling of palms for a new power line the previous year, the first infected beetles were collected from traps 15 weeks after virus release. At Keravat also, marked infected beetles were released in a block of coconuts heavily damaged by *O. rhinoceros*, and unmarked infected beetles were first recovered 10 weeks later. At Vuvu, the first positive beetle was trapped within the release site 21 weeks after virus release, and other infected beetles were caught in traps 2·0, 3·3, 4·5 and 5·7 km from the release site.

The rate of spread of virus in the field was found to be approximately 1 km/month. This estimation was based on three separate assessments: at Vuvu, where infected beetles were collected 5·7 km from the release site after 21 weeks; at Tokua, where the infection spread 6 km over a 22-week period; and at Vudal College where an infected beetle was collected 3 km from the release site at Keravat after 13 weeks.

Female beetles were more frequently ($P<0{\cdot}001$) caught than males during the seven fortnightly trapping periods between 9 July 1979 and 14 October 1979 (Table II). In addition, a higher proportion of males (24%) than females (11·5%) were infected. This difference was not significant ($0{\cdot}05<P>0{\cdot}1$, by χ^2), but was significant by Friedman two-way analysis of variance by ranks ($P<0{\cdot}001$).

From collection from Manus Island on 14–20 August 1979, it again appeared that males become infected more readily than females, but the difference in infection rates was less marked than that from the Gazelle Peninsula: 17 of the 19 males were positive (89%) compared with 13 of 18 females (72%).

TABLE II. *Numbers, sex and virus incidence of* O. rhinoceros *adults caught in traps in fortnightly periods on the Gazelle Peninsula from 9 July 1979 to 14 October 1979*

Fortnight beginning	Females			Males			Percentages infected	
	+ve	−ve	Total	+ve	−ve	Total	Females	Males
9 July	2	25	27	10	11	21	7·4	47·6
23 July	0	34	34	0	14	14	0·0	0·0
6 Aug.	0	28	28	0	12	12	0·0	0·0
20 Aug.	5	28	33	6	12	18	15·1	33·3
3 Sept.	8	22	30	1	11	12	26·6	8·3
17 Sept.	3	13	16	3	10	13	18·7	23·1
1 Oct.	3	12	15	5	9	14	20·0	35·7
Total period	21	162	183	25	79	104	11·5	24·0

+ve = virus positive
−ve = virus negative.
Differences in sex frequencies in fortnightly catches by χ^2 are significant ($P<0{\cdot}001$).
Differences in virus incidence between sexes by Friedman two-way analysis of variance by ranks are significant ($P<0{\cdot}001$).

Trap catches remained fairly constant in the eight months after the first virus release on the Gazelle Peninsula and there was no indication of a decline in the numbers of beetles caught.

Palm damage surveys

By November 1979, there had been no indication of a decrease in the amount of damage to palms by *O. rhinoceros*.

Discussion

The pre-release studies indicated that the baculovirus of *O. rhinoceros* did not occur naturally in Papua New Guinea. This evidence was based on histological studies of more than 400 adults and from more than 2000 larvae, collected from all three regions in Papua New Guinea where the beetle is present. Both Bedford (1973*a*) and Zelazny (1977*b*) had stated that the virus was not found in Papua New Guinea, but presented no evidence for these statements.

The virus was only recovered from the field after the controlled release of the baculovirus during 1978 and 1979. Virus imported from Western Samoa proved highly infectious to both larvae and adults of the local population of *O. rhinoceros*.

The smear technique proved valuable in the diagnosis of virus infections in the beetles. With practice, it became relatively easy to distinguish epithelial nuclei and to make accurate diagnoses from smears.

Guts that appeared unusual on dissection were often processed into both serial sections and smears. When material prepared by both methods was examined, the diagnoses were nearly always identical.

The recovery of unmarked infected beetles from the field indicated that the virus became established in the local population. This occurred readily despite the much smaller numbers of infected adult *O. rhinoceros* that were released in Papua New Guinea, compared to the large numbers that were released in Fiji (Bedford, 1976*b*). A good example was at Kalili in New Ireland where only 27 adults were infected and released. All of these had been collected from breeding sites, and a large proportion would be expected to be teneral and thus less likely to succumb to the virus disease after inoculation (W. D. Paul, pers. comm.). However, 24 weeks later, five out of ten unmarked beetles caught in traps were infected.

The trapping method could be used to give an approximation of the general virus level in the population, even though it was not applied randomly. Care was taken to ensure that cross-contamination did not occur between beetles caught in different traps, thus ensuring that the percentages of infection observed in trapped beetles, reflected more accurately the situation in the field population.

Young (1974) found a rate of spread of virus in Tonga of 3 km/month. However, the rate of spread in Papua New Guinea of approximately 1 km/month, cannot be compared directly with that of Tonga, where an entirely different monitoring system, examination of breeding sites, was used.

The differences between the proportions of each sex of the beetle caught in traps agrees with that observed by Bedford (1973*b*). Zelazny (1977*a*) also noted a higher incidence of virus infection in males in Western Samoa.

The establishment of the baculovirus disease of *O. rhinoceros* in Papua New Guinea leads to the hope that this rhinoceros beetle will be successfully controlled as it has been in other countries.

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