The *Oryctes* virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Abstract

In view of the increasing and devastating damage by rhinoceros beetle (*Oryctes rhinoceros*) to coconut palms in the middle of last century, many efforts were made to find an efficient natural control factor against this pest, which could not be controlled by pesticides. The basic procedures of these monitoring programmes are outlined together with the final detection of a virus disease in oil palm estates in Malaysia in 1963. In extensive laboratory studies, the virus was isolated and identified as the first non-occluded, rod-shaped insect virus, morphologically resembling the baculoviruses. Infection experiments clarified the pathology, histopathology, and virulence of the virus and demonstrated that the virus was extremely virulent to larvae after peroral application. These findings encouraged the first pilot release of virus in 1967 in coconut plantations of Western Samoa where breeding sites were contaminated with virus. Surprisingly, the virus became established in the Samoan rhinoceros beetle populations and spread autonomously throughout the Western Samoan islands. As a consequence, there was a drastic decline of the beetle populations followed by a conspicuous recovery of the badly damaged coconut stands. This unexpected phenomenon could only be explained after it was shown that the adult beetle itself is a very active virus vector and thus was responsible for the efficient autodissemination of the virus. The functioning of the beetle as a 'flying virus factory' is due to the unique cytopathic process developing in the midgut after peroral virus infection. Pathological details of this process are presented. Because of the long-term persistence of the virus in the populations, rhinoceros beetle control is maintained. Incorporation of virus into integrated control measures and successful virus releases in many other countries are recorded.
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1. Introduction

Thanks to the persuasive arguments of Dr. Trevor Jackson it was possible to reactivate a Methuselah like me from retirement to report on the discovery and history of the *Oryctes* virus, and its implementation in biological control of its host, the coconut palm rhinoceros beetle, *Oryctes rhinoceros*. In a paper by Caltagirone (1981), this successful practical use of an insect virus was ranked as a landmark in classical biological control. There is a series of papers from the 1960s to 1970s relating to the successful release and colonization of this virus followed by the collapse of rhinoceros beetle populations, virus persistence, and long-term pest control. Yet in certain areas little attention has been paid to the fact that the optimal control capacity of the virus can only be secured by complying with other parameters of an integrated control program. The creation of a local

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abundance of breeding sites, as often happens during palm clearing measures for agricultural, industrial, and housing programmes, may disrupt the balance of control. The historical success of this program, and threats to its failure if IPM factors are neglected, make it worthwhile to refocus attention on the *Oryctes* virus in this SIP symposium.

2. History of the Oryctes virus

2.1. Conditions and search for natural control agents in the 1950s around the tropic belt

Looking at the history of the *Oryctes* virus, we have to recall the years around the middle of the last century. At that time, the rhinoceros beetle caused increasing devastating damage to coconut palms in many regions, especially in the South Pacific, where the pest had accidentally been introduced. Thus, in the Palau Islands, about 50% of the coconut palms were destroyed by the beetle within 10 years of its introduction (Gressit, 1953). Since the coconut palm, the so-called 'Tree of Life,' is of outstanding importance as a subsistence and export crop in large areas of the tropics, many efforts were made to abate this serious situation. At that time, chemical control was in its euphoric phase in general plant protection, though Rachel Carson (1962) had already alarmed the public of the negative consequences in her visionary book 'Silent Spring.' Of course, various attempts had been made to control the rhinoceros beetle by pesticides. In the Fiji Islands, for instance, leaf axils of palms were furnished with sawdust contaminated with pesticides to prevent the beetle from spreading. Yet, this very laborious and costly procedure was also in vain. As a matter of fact, due to specific factors in its biology and ecology, the beetle cannot efficiently be controlled by pesticides. Neither the larval broods feeding in scattered decomposing organic matter nor the adults, visiting these breeding sites or hiding in their feeding burrows in the central spear of palms, are readily accessible to chemical control.

Therefore, the search for effective natural control agents always was of special interest and was intensified in the 1950s and 1960s. Dr. Paul Surany, engaged by the former South Pacific Commission, carried out an extensive survey of possible diseases of *Oryctes* spp. in many countries around the tropic belt from 1955 to 1959. In his compilation of results (Surany, 1960), a vast amount of probable pathological conditions was described and classified, especially under two presumed diseases, the so-called Heidenreich's disease and Maya's disease. Unfortunately, in both cases Koch's postulates were not accomplished. Thus, an irrefutable proof that these abnormal conditions found in *Oryctes* spp. were caused by some pathogen is still pending.

2.2. Successful search for natural control agents in the 1960s

Under these circumstances and in view of the continuous severe palm damage caused by *O. rhinoceros* in the South Pacific, the South Pacific Commission decided to continue the diagnostic surveys to find an efficient disease agent that could be used in biological control of this pest. In 1963, I agreed to a 4-month contract to carry out these studies. In putting up my working schedule I felt that I had to change the strategy of search practised to that date. To increase the chance of success in this challenging task, I did not envisage surveys in many regions of the tropic belt. Also, I did not focus my activities on the South Pacific islands where the beetle had accidentally been introduced and where damage to palms was greatest. Rather I decided to concentrate my survey studies primarily on South-east Asia, specifically Malaysia including Borneo. This decision was made from the simple reason that South-east Asia is an autochthonous area of *O. rhinoceros*, which, from general experience, should offer the greatest chance of detecting an effective natural control factor.

The survey was carried out with the intention of investigating as many broods and colonies of the beetle as possible all over the country. For this purpose, the single standing dead oil palm trunks scattered in older plantations and rotting from the top offered an ideal source for a widespread examination of larval broods. Most of the felled trunks harboured more or less numerous larval colonies in their rotting top. As usual during the whole survey, specimens with suspicious signs and symptoms of disease were collected and dissected in the laboratory for examination of tissue squash preparations in phase contrast.

An extremely rich supply of all developmental stages of the rhinoceros beetle was always available in the replanted plots of oil palm plantations. Oil palms are usually replanted after 30–35 years. In this process, masses of felled palm logs remain lying next to the replanted young palms and are gradually decomposing from top to base. In this way, the rotting logs, and later also the stumps, offer an abundance of breeding substrate for the rhinoceros beetle, as long as they are not hidden by a leguminous ground cover. To protect the young oil palms from deadly beetle attack, at that time so-called beetle gangs regularly chopped up the rotting portions of the logs and stumps, and collected all beetle stages they found. Their collections also nicely contributed to my survey in which extensive diagnostic studies were carried out (Huger, 1966a).

After 8 weeks of intensive monitoring activities, the first larval samples with striking signs and symptoms of disease were encountered: suspect larvae collected from some oil palm estates and kept in buckets in a field insectary, surprisingly developed a negative geotropism and came up to the surface of the feeding substrate where

they remained lying (Fig. 1). They were extremely lethargic and had totally changed their usual appearance. The fat body could be seen through the integument in a progressive stage of dissolution and disintegration, especially in the abdominal region (Fig. 2). The suspect larvae often attained a sort of dropsical condition with an increase of hemolymph. In this stage, they were fairly translucent when viewed against a near light source. In the final phase of disease, most larvae appeared shiny, beige and waxen and, not infrequently, their turgidity was increased, even to the degree that their rectum completely prolapsed (Fig. 3B). Often in this terminal stage of disease, the larvae displayed chalky-white accumulations under the integument (∼0.5 to 4.0 mm in diameter) in a whitish mottled pattern. In phase contrast, squash preparations of the disintegrated fat body showed myriads of tiny particles in rapid Brownian movement.

Fig. 1. Historical photo displaying the first virus-diseased third instar larvae of the rhinoceros beetle detected in Malayan oil palm estates in 1963. The heavily diseased larvae are lying on the surface of the feeding substrate.
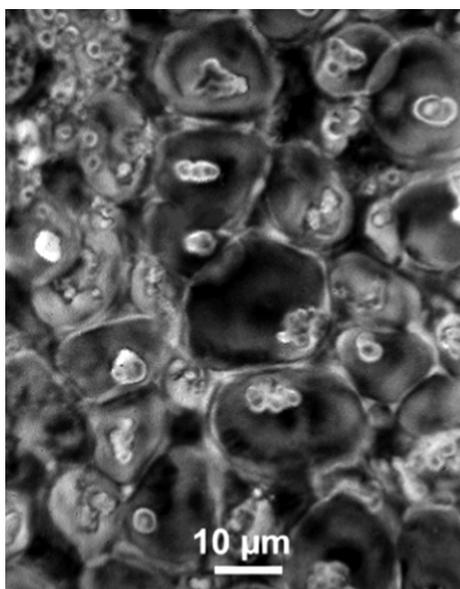
Fig. 2. Massive disintegration and vacuolation of fat body tissue of a virus-infected third instar larva of the rhinoceros beetle in phase contrast.
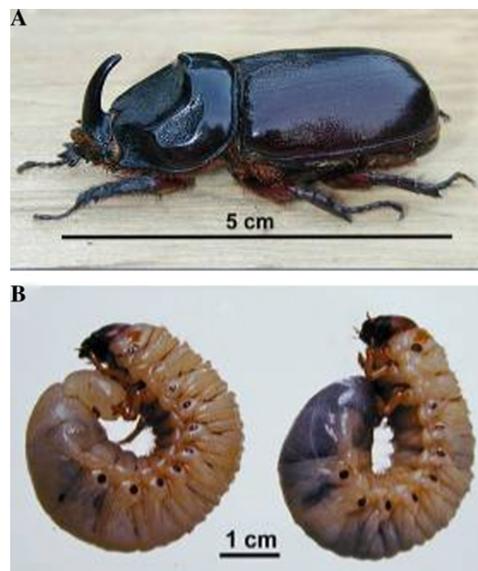
Fig. 3. (A) Male adult rhinoceros beetle. (B) Third instar rhinoceros beetle larvae. Left: With heavy signs of virus infection including prolapse of rectum. Right: Healthy specimen.

As all signs and symptoms of these larvae clearly indicated that they had been suffering from a genuine disease, a representative larval sample was sent by air to my home laboratory in Darmstadt, where the material was deep-frozen, for detailed later studies. Final searches for new diseases of the rhinoceros beetle were carried out for some weeks in Fiji and in (then) Western Samoa without any important findings.

2.3. Basic research on the Oryctes virus: its identification and description

After returning to Darmstadt, a rearing of the rhinoceros beetle was established with specimens shipped from Malaysia. The indigenous *Oryctes nasicornis* was also reared for comparative studies. The first steps in basic research were aimed at clarifying the etiology of the disease phenomena of the larvae then collected in Malaysia and stored so far in the deep freezer. For this purpose, peroral infection experiments were carried out with healthy 3rd instar rhinoceros beetle larvae by feeding them rotting sawdust contaminated with a suspension of triturated diseased larvae. Surprisingly, all larvae developed the same signs and symptoms of disease observed in Malaysia. They died within 1–4 weeks and were also lying on the surface of the substrate in the final phase of disease. Investigations of ultrathin sections of fat body and other tissues from both experimental and deep-frozen larvae revealed the disease to be caused by a free rod-shaped virus being reproduced in the nucleus (Fig. 4). From these results, Koch's postulates had been accomplished.

In size and structure, the *Oryctes* virus very much resembled the baculoviruses of the many nuclear polyhedrosis and granulosis diseases of insects known at
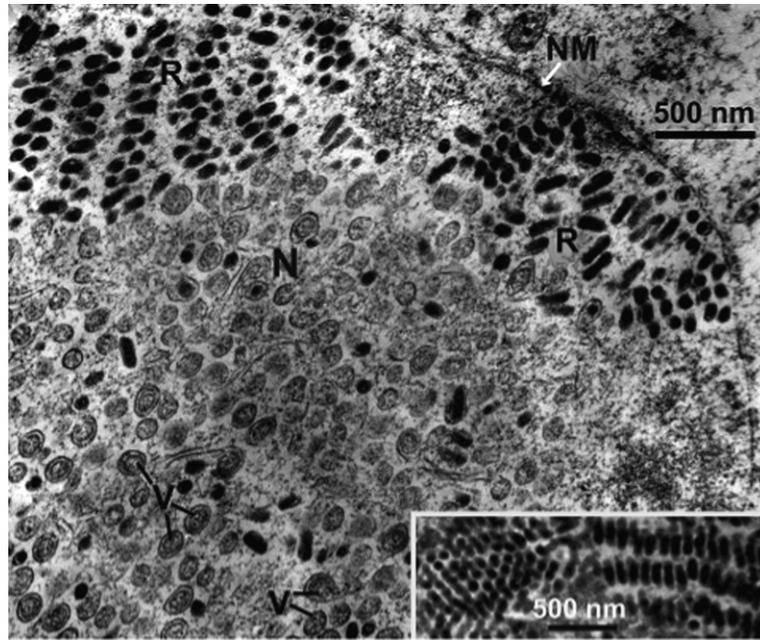
Fig. 4. Electron micrograph of a thin section showing part of the nucleus (N) of a fat body cell of a third instar rhinoceros beetle larva with heavy virus infection. Note the accumulation of virus rods (R) at the nuclear periphery and the single- and double-membraned vesicles (V) in the nuclear center. NM, nuclear membrane. Inset: Virus rods in cross (left) and longitudinal (right) section arranged in a pseudocrystalline pattern.

that time. But the main difference was that, with minor exceptions, it is not occluded in paracrystalline proteinaceous bodies (Huger and Krieg, 1991). Therefore, this first rod-shaped, non-occluded insect virus was assigned to a new genus and originally described as *Rhabdionvirus oryctes* (Huger, 1966b). When the classification and nomenclature of insect viruses was completely revised by the International Committee on Taxonomy of Viruses (ICTV), the *Oryctes* virus became the type species of Subgroup C of the family Baculoviridae. Thus, for a long time it was cited in literature as 'baculovirus of *Oryctes*' or briefly as '*Oryctes* baculovirus.' Recently, Evans and Shapiro (1997) have assigned the virus to a new *Oryctes* virus family.

During reproduction of the virus in the hypertrophied nuclei, an abundance of vesicular virus envelope material (ø ca. 160 nm) is produced (Fig. 4). The virus rods tend to accumulate in the marginal area of the nucleus forming the so-called ring-zone, where they may also be densely packed in a two- or three-dimensional pseudocrystalline pattern (Fig. 4) (Huger, 1966b). Depending on the isolation procedure, negatively stained virions measure from about 200 to 235 nm in length and 100 to 120 nm in width; the mean size of the nucleocapsids is 180×65 nm (Fig. 5). In thin sections, the average size of the virus rods is 195×70 nm.

The many infection experiments carried out invariably showed that the virus is very infective and virulent to all larval stages, so concepts were developed for a pilot virus release experiment in Samoa. The idea was to contaminate artificial breeding sites in the coconut plantations
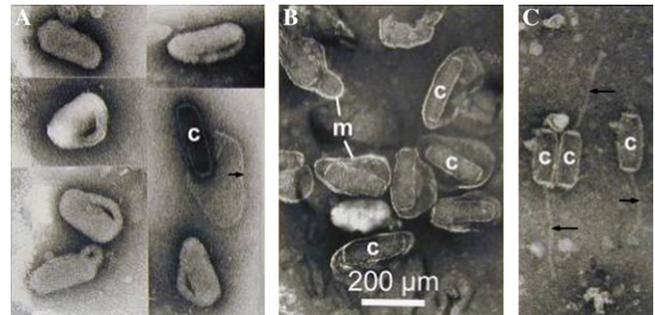
Fig. 5. Electron micrographs with structural details of *Oryctes* virus rods, negatively stained with phosphotungstic acid. (A) Virions unpenetrated by stain, often being artificially mug-shaped; middle right: the virus membrane (arrow) is shed off from the capsid (c). (B) Virions with longer penetration by stain, thus displaying the capsids (c) and the surrounding viral membrane (m). (C) Three capsids (c) showing the typical thread-like appendix (arrows).

with a suspension of triturated diseased larvae, so that the virus might colonize in the rhinoceros beetle populations.

2.4. Pilot virus release experiment in Samoa

For the execution of these pilot virus release experiments, Dr. K.J. Marschall was trained in our laboratory. He joined the new *Oryctes* project of the South Pacific Commission, which was established in Samoa in 1964 with FAO assistance to find some way of limiting this serious pest. The project was initially supplied with virus by shipping infected larvae from our laboratory in Darmstadt to Samoa.

In view of the abundance of rhinoceros beetle larvae in Samoa and their susceptibility to the virus, it was easy to produce the necessary amount of virus for release in the field: large numbers of larvae were just fed with rotting sawdust contaminated with triturated diseased larvae.

In 1967, the first pilot virus release experiment was carried out in Samoa. Rotting sawdust contaminated with virus was distributed over heaps of rotting coconut logs, an ideal breeding substrate for the rhinoceros beetle. In this way about 1500 triturated dead infected larvae were distributed on the island of Manono and in two locations on Savai'i. About 18 months later, larvae with symptoms of virus disease were collected on Upolu, where no virus had been applied. Smaller numbers of larvae with symptoms of virus disease were found on Savai'i, while on Manono the beetle population had already collapsed (Marschall, 1970). By infection experiments and electron microscopy in our Darmstadt laboratory it was confirmed that the Samoan field-collected diseased larvae had been infected with the typical rod-shaped *Oryctes* virus released earlier.

Surprisingly, the virus disease continued to spread autonomously over the Samoan islands, and at the same time there was a drastic collapse of the beetle populations. As a consequence, the coconut palms nicely recovered from damage by the rhinoceros beetle and allowed recovery of the copra industry. Obviously an effective virus vector was responsible for the autodissemination of the virus over large areas. Evaluating the question of virus vectoring, the adult beetles came under suspicion (Fig. 3A). When infection experiments showed that rhinoceros beetle adults were also perorally highly susceptible to the virus and, in addition, develop a unique cytopathic process in their midgut, it became clear that the beetles themselves function as very efficient natural vectors of the disease.

2.5. The unique disease process in rhinoceros beetle adults and their role in the autodissemination of the virus

At an early stage of adult infection, virus reproduction takes place in the dense hypertrophied nuclei of the midgut epithelium. This pathological condition stimulates the crowds of adjacent regenerative crypts to start massive cell proliferation in their apical region (Fig. 6). Due to this enduring vigorous cell proliferation, the midgut lumen is completely filled up with a dense accumulation of cells within 1–2 weeks, as shown in Fig. 7. All the hypertrophied nuclei of this myriad of cells are heavily infected and packed with virus (Fig. 8) (Huger, 1972; Huger and Krieg, 1991). In this way, enormous amounts of virus are produced by each beetle; it is estimated to amount up to 0.3 mg per day (Monsarrat and Veyrunes, 1976). Dissected infected beetles show the midgut to be greatly extended
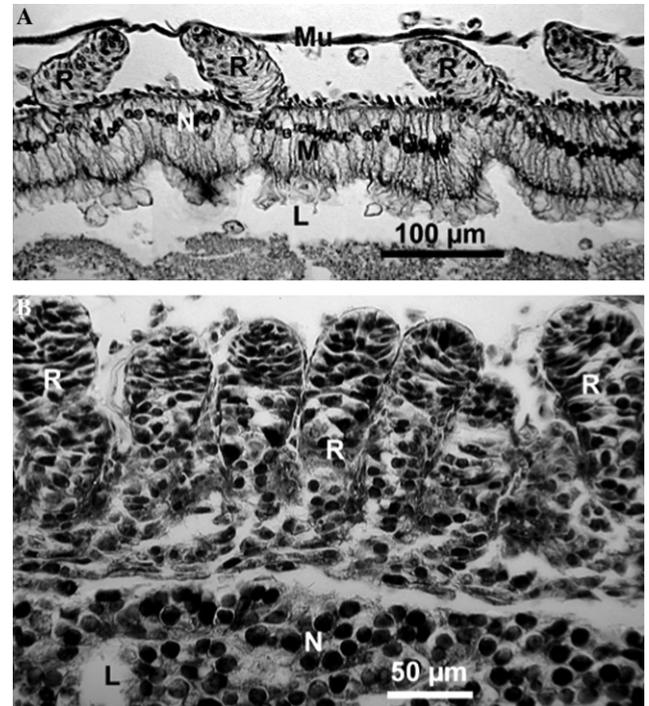
Fig. 6. Light micrographs showing the midgut epithelium of rhinoceros beetle adults in longitudinal sections stained with Heidenhain's hematoxylin. (A) Midgut epithelium (M) of a healthy adult with a black-stained row of cell nuclei (N); the epithelium is outside occupied by regenerative crypts (R) and bounded by the muscularis (Mu); L, midgut lumen. (B) Midgut epithelium of a virus-diseased adult with greatly enlarged regenerative crypts (R); the latter are massively proliferating cells from their apical region into the midgut lumen, where the hypertrophied cell nuclei (N) produce large amounts of virus.
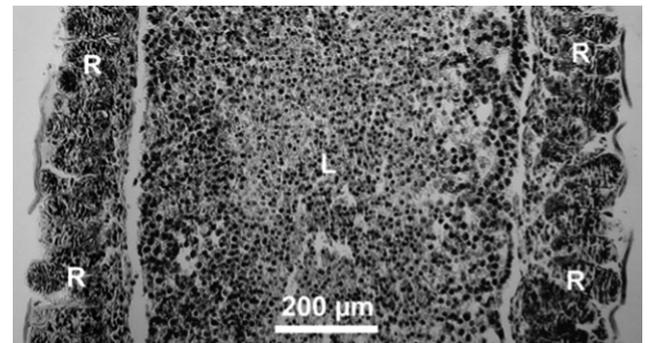
Fig. 7. Light micrograph of a longitudinal midgut section of a heavily virus-diseased rhinoceros beetle adult, stained with Heidenhain's hematoxylin. By massive cell proliferation of the regenerative crypts (R), the midgut lumen (L) is completely filled up with cells, the nuclei of which appear black-stained.

and swollen, with a purulent appearance as compared to the relatively thin midgut of healthy specimens.

For many weeks, such infected beetles are flying around and defecating large quantities of virus into their natural habitats, i.e., in the widely scattered breeding sites and in the feeding burrows of palm crowns, so that other individuals including larvae may catch the infection. In this way they provide an effective horizontal
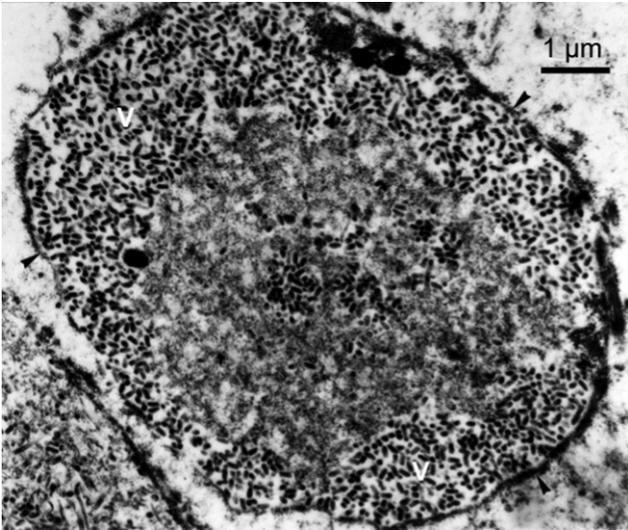
Fig. 8. Electron micrograph of a thin section through a nucleus from the myriads of cells in the midgut lumen of a virus vectoring rhinoceros beetle adult. The rod-shaped virions (V) are accumulating in the marginal 'ring zone' of the nucleus, while virus assembly is progressing in the nuclear center. Arrowheads show nuclear membrane.

virus transmission in a kind of snowball system. Most frequently, virus is transmitted directly to other adults by oral contact with fecal virus during mating or by co-occupation of the same habitat (Zelazny, 1976). Therefore, the chronically infected adult beetles represent very productive flying virus reservoirs that disseminate virus in an efficient and ideal manner. The unique cytopathic process in the midgut of adults and their role in extended spatial virus colonization finally explained the epizootic breakdown of rhinoceros beetle populations upon initial virus release in Samoa. The drastically reduced fecundity of infected females greatly contributes to such population declines (Zelazny, 1973, 1977).

2.6. Virus releases in other countries

With the success of the Samoan virus releases, subsequent releases have been conducted in many countries. For the initial releases, the virus was distributed by the method practised in Samoa. As soon as the role of virus vectoring by adults was disclosed, a far more simple and economical method of virus release was employed: Adults were trapped in tin boxes on a console fixed to palm stems. The tin boxes were covered with a slice of a coconut stem furnished with a hole in the centre. A small quantity of the attractant ethyl chrysanthemumate was applied on the underside of the wooden slice. The collected trapped adults were infected with virus by applying a drop of virus suspension onto their mouthparts or by forcing them to swim for 10 min in a 10% suspension of freshly triturated virus-diseased larvae. After 1–2 weeks, beetles treated in this way were released in the field to initiate the epizootic process.

Following Samoa, virus releases with subsequent conspicuous reductions in palm damage of up to 95% were carried out in the South Pacific area, e.g., Fiji, Tonga, Wallis Island, Tokelau Islands, Palau Islands, and American Samoa (for literature, see Bedford, 1980). Further, successful virus releases took place in Papua New Guinea (Manus Island, New Ireland, and New Britain) (Gorick, 1980), Mauritius (Monty, 1978), the Maldives (Zelazny, 1990), Oman, and other places. Although *Oryctes monoceros* is less susceptible to the virus, the virus was also released against this pest in the Seychelles, with a modest population reduction of ca. 30% (Lomer, 1986), and in Tanzania (Purrini, 1989).

3. Concluding remarks

The autonomous epizootic spread of the *Oryctes* virus upon release leading to drastic decline of rhinoceros beetle populations below economic levels and long-term persistence of the virus with maintenance of control indicates this was the "classical" method of biological control. "Classical biological control" has been defined as a single introduction and autodistribution that is sufficient to bring the target pest under control (Caltagirone, 1981). For instance, in Samoa where the virus was released in 1967, the rate of persistent infection of adults fluctuated between 27 and 45% during the period 1976 to 1983 (Stechmann and Semisi, 1984). According to long-term experience in many virus release areas, such infection rates suffice to keep the populations under the economic threshold. However, this stable situation may be severely disturbed when people forget that the virus is just a component—though the primary and most effective one—in an integrated control program for the beetle (Huger, 1966a, 1978). In this situation, plantation hygiene, i.e., sanitation measures to eliminate local breeding sites, is of primary importance. People, particularly governmental plant protection services, are well advised to continue removing breeding sites. This will conform with the principle of 'cumulative returns' in virus dissemination and prevent the resurgence of rhinoceros beetle populations. The virus will then be able to maintain permanent suppression of rhinoceros beetle, the most serious pest of coconut palms in the Asia/Pacific region. As yet there is no evidence that rhinoceros beetle populations are developing resistance to the virus.

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