Biology and Management of Palm Dynastid Beetles: Recent Advances

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Annu. Rev. Entomol. 2013. 58:353–72

The *Annual Review of Entomology* is online at ento.annualreviews.org

This article's doi:
10.1146/annurev-ento-120710-100547

Keywords

Oryctes, Nudivirus, *Metarhizium*, palms, pheromone, *Scapanes*

Abstract

Coconut, oil, and date palms are important crops in the tropics and are attacked by dynastids that cause loss of production or death of hosts. Knowledge of their breeding sites has been extended since a previous review in 1980. The fungus *Metarhizium anisopliae* has potential as a biopesticide against immature stages in friable breeding sites. The molecular biology and ultrastructure of *Oryctes rhinoceros* Nudivirus (OrNV), disseminated by adults, have been studied, and this pathogen can reduce *O. rhinoceros* populations and damage when introduced into new locations, especially where damage had been high. New PCR techniques may enable reliable quantification of dosages ingested and hence virulence of different isolates. Male-produced aggregation pheromones have been identified in several species, for which they may have management potential, having been used commercially for trapping *O. rhinoceros* in oil palm plantations in Southeast Asia, and tested against *O. monoceros* in Africa.

Supplemental Material

INTRODUCTION

Coconut, oil, and date palms are attacked by various species of rhinoceros beetles (Coleoptera: Scarabaeidae: Dynastinae) (**Supplemental Figure 1**, follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). This damage directly causes loss of production or death and can lead to lethal secondary infestation by palm weevils (*Rhynchophorus* spp.). Attacks on date palms by larvae may cause the tree to fall over. Work conducted up to approximately 1980, including the zoogeography of these dynastids, has been reviewed elsewhere (10). This review covers work since then, though I refer to earlier work as necessary. Taxonomy of the Dynastinae has been reviewed (25). Coconut palms may be planted in large commercial plantations, in smaller groups, or as individuals in villages or gardens as food sources or ornamentals, and each of these ecosystems has its own characteristics, with consequences for beetle population dynamics and management. Oil palms appear in regular plantations only. All studies mentioned relate to managed palm plantings.

BIOLOGY OF IMMATURE STAGES

Palm dynastids utilize a diversity of breeding sites where immature stages and adults may be found (**Supplemental Figures 2 and 3**). *Oryctes rhinoceros* is very versatile in the variety of sites used, taking advantage of newer types of breeding sites, such as shredded oil palm trunk material in Malaysia (66, 68) or debris in axils of coconut fronds in Guam (99), in addition to well-known sites, such as dead palm poles and trunks (**Supplemental Figures 4 and 5**) and cow-dung and compost heaps. This adaptability underlines the risk of *O. rhinoceros* establishment should it reach new regions. The immature stages are cryptic inside breeding sites that may occur within, or outside, areas of palms damaged by adults. The study of immature numbers may thus be separate from that of adults, and sampling larvae disturbs the ecosystem, making population studies difficult, particularly in areas where coconut palms are grown (106). Further details of the range of palm dynastid breeding sites and the occurrence of endemic entomopathogens at various locations are shown in **Supplemental Table 1**.

A key for separating larvae of palm pests from other types of larvae that coexist with them in breeding sites in Papua New Guinea has been presented for field use in areas where the species are known to occur (8, 9). Ecological criteria could be incorporated into it, such as the absence of *Scapanes australis* larvae in the tops of dead standing coconut poles (10). A detailed morphological determination would be essential if important pest dynastids were suspected of extending their distributions into new geographic areas.

MANAGEMENT OF IMMATURE STAGES BY *METARHIZIUM ANISOPLIAE* FUNGUS

Pathogenicity and Production

Metarhizium anisopliae can be long- or short-spored, with the long-spored (*M. anisopliae* var. *major*) varieties isolated from *Oryctes* spp. being more virulent (78); four such Malaysian isolates killed all third-instar larvae after 14 days (78, 79). The infective potential of a strain may become reduced by culturing on media but restored considerably following infection of a host (26). However, scarabaecin, a chitin-binding antifungal peptide, has been isolated from *O. rhinoceros* hemolymph (97).

Methods for mass-producing spores (20, 81, 95, 96) result in a powder, in which spores remained approximately 60% viable after 15 months when stored at an optimum temperature of 5–15°C (79).

In laboratory trials, granule formulations incorporating mycelium and nutrient-growth medium, or spores only, infected 93% of third-instar larvae in rotting oil palm material 18 days after treatment, whereas water suspensions of spore powder killed after 12–14 days (82). For natural incidence studies (**Supplemental Table 1**), specimens must be held individually from the moment they are collected from the field—if bulked together, one infected specimen will most likely infect all.

India

In Kerala, *M. anisopliae* and OrNV (*Oryctes rhinoceros* Nudivirus) coexist in *O. rhinoceros* breeding sites (**Supplemental Table 1**). *M. anisopliae* infection is most frequent when rainfall and humidity are high; the spores should be applied to breeding sites as a biopesticide then, when the possibility of OrNV transmission is at its lowest, thus avoiding conflict between the two pathogens (30). In vermicomposting sites, treatments with *M. anisopliae* spores killed all third-instar larvae, with the highest dose giving the fastest kill, taking 8 days when favored by high humidity (31).

Nudiviruses: arthropod-specific rod-shaped enveloped circular double-stranded DNA–containing viruses

Biopesticide: a microorganism with potential for propagation and use as a pathogenic IPM agent against an insect species

OrNV: *Oryctes* Nudivirus

Malaysia

Heaps of rotting oil palm trunk residues under a leguminous cover crop were sprayed with suspensions of spores, which caused mycosis levels of 30–33% after 8 months, compared with control heaps with 13% mycosis. By 12 months, infection levels for treatments were approximately 70%, mostly for third-instar larvae, and 52% for control heaps (81). There was no reduction in adults caught in pheromone traps in the study area because they apparently immigrated from surrounding untreated areas. These results indicated that *M. anisopliae* is best applied early, i.e., 6 to 8 months after chipping of the trunks; the water carries the spores deeper into the breeding sites and aids their germination, and the mycosis replenishes the spores (78). Without spore applications, two-year-old shredded rotting coconut trunk debris, with 0–2% *M. anisopliae* infections and cover crop overgrowth, could contain substantial numbers of larvae (96).

BIOLOGY OF ADULTS

Oryctes rhinoceros

The adult (**Supplemental Figure 1**) bores into the crown or heart of the coconut palm (**Supplemental Figure 6**), or into the base of the cluster of unopened fronds (spear) of young oil palms, damaging several of the still-furled fronds as it feeds on the sap. The damaged fronds show characteristic V- or wedge-shaped cuts as they unfold, reducing the photosynthetic area (**Supplemental Figure 7**). Severe repeated attacks kill the growing point, resulting in the death of the coconut palm (**Supplemental Figure 4**). The effects of damage can be much more harsh, often lethal, on young oil palms (less than a year old) than on more mature ones (104).

Studying the biology of *O. rhinoceros* adults is difficult (106). They are cryptic, hidden in their feeding holes or in breeding sites, and fly only at night. Sampling by cutting them out of feeding holes or breeding sites, or by trapping, disturbs the ecosystem and population size at the study sites. Breeding sites may occur in areas of palms where adults feed, or they may be distant from or outside the feeding area. Different proportions of the adult population may be present in the two habitats. Adults may be present in low densities even in severely damaged areas, and density may vary widely, being related to density of palms and breeding sites (106). In regular plantations, there may be low or negligible damage at the center, and severe damage at the edges due to adults flying in from outside the planting. Because a coconut palm may reach a height of 30 m, and the structure of its crown is complex with a radiating head of fronds, a new feeding hole cannot be seen from the ground and adults may enter palms while others leave on a daily basis.

EC: ethyl chrysanthemumate

E4-MO: ethyl 4-methyloctanoate

Sensilla: organ for detecting sensory stimuli

Rachis: the principal axis of a compound leaf or frond

For these reasons, there had been no detailed accounts of population density (106) at the time of the 1980 review (10); however, in New Britain, Papua New Guinea, an average immigration rate into a study site of young palms was determined (10). Rather than attempting to measure the population directly, as the damage becomes obvious as the fronds open (at approximately 1 frond per month; 111), methods for measuring damage as an index of the population were developed (105). A detailed damage survey gives a percentage of fronds damaged in a sample of palms, whereas a rapid damage survey gives the percentage of palms damaged in the central three to five fronds, i.e., the most recently opened (examples of earlier studies using these methods are given in Reference 10). If further data were required, by numbering fronds, starting from the central spear leaf as 0 and noting the position of each cut (111), a series of calculations would lead to an estimate of the number of feeding adults present per month, with one attack lasting an average of 5 days, but searching breeding sites for adults is needed to confirm such estimates, which disturbs the ecosystem of the study area for further observations. In the Philippines, the life span of an adult is approximately 15 weeks, including 3.5 weeks at the pupation site after eclosion (111).

In South Luzon, Philippines, 7–25% of adults caught over 3 years in traps baited with the synthetic attractant ethyl chrysanthemumate (EC) (**Supplemental Table 2**), and 0–20% feeding in palms, were infected with OrNV, and the percentage of females trapped rose and fell in parallel with the incidence of OrNV (112). In Kerala, India, incidences of OrNV infection in adults from palm crowns were 41–75% (58) and 60% (23), and the incidence of infection in adults from breeding sites was 22% (30).

Ethyl 4-methyloctanoate (E4-MO) was first found in Indonesia to be the major aggregation pheromone component produced by males (35, 62, 90). Its occurrence in different samples was irregular, so it is likely that males produce E4-MO only under specific conditions (62). Olfactory receptor neurons responsive to E4-MO were found in sensilla in male and female antennae (85). See **Supplemental Table 2** for further details of palm dynastid pheromones.

Oryctes monoceros

Coconut palms and seedlings were damaged by *Oryctes monoceros* in the Republic of Seychelles (49), and in Nigeria approximately three adults were removed per month from 3-year-old oil palms (98) and it attacked lower-height coconut palms during the rainy season (1). In the Ivory Coast, in the laboratory both sexes, but particularly males, were active between 0800 and 1400 hours (3), whereas *O. rhinoceros* is customarily regarded as nocturnal (33). E4-MO is the male-produced aggregation pheromone (34).

Oryctes agamemnon

In Tunisia, nocturnal adults of *Oryctes agamemnon* feed on soft wood at the crown of date palms (47), causing subsequent rotting, but other investigators (93, 94) found little sign of feeding during laboratory rearing. In Saudi Arabia, they attacked fruit stalks and the rachis of fronds, and in Oman, light-trap catches over three years peaked during the driest warmest season (5).

Oryctes elegans

In eastern Iran, *Oryctes elegans* adults tunnel into date frond bases and stalks of fruit bunches and are strong fliers, rapidly colonizing new plantings (86). Near Baghdad, the species is univoltine and adults are caught in light traps from July to October (an average of 29 adults trap^{-1} month^{-1}) (46). The male-produced pheromone is 4-methyloctanoic acid (86).

Scapanes australis

Scapanes australis (**Supplemental Figure 8**) attacked oil palms at Popondetta, Papua New Guinea (89) (**Supplemental Figure 9** shows typical damage from New Britain), and young coconut palms in Melanesia (**Supplemental Figures 10 and 11**). In East New Britain, the immigration rate into blocks of young coconut palms fell away as the palms aged (10). When placed into an artificial feeding gallery in a young coconut palm, a male *S. a. grossepunctatus* would come to the entrance and poke out its abdomen and emit a liquid secretion containing an aggregation pheromone consisting of three components (87, 88) (**Supplemental Table 2**), which was then smeared by the hindlegs ("calling"). This pheromone attracted both sexes. Arriving females lacked developed eggs and mated with the resident male. Arriving males displayed aggressive behavior, fighting with the resident male for possession of the gallery (74). A male with sugar cane in a trap also attracted both sexes (43). Radio-telemetry studies in Madang, Papua New Guinea, showed that flights of *S. a. australis* were nocturnal. Males flew within 5 m of the ground erratically for 52 to 835 m per flight, whereas females flew in a tight upward spiral above the coconut canopy (>20 m) and then in a straight path for 245 m to less than 1 km per flight, in one case to a feeding gallery made by a male, thus colonizing newly planted palm blocks (7). This difference in flight and dispersal behavior between the sexes may correlate with the observation that, although both sexes of *S. australis* subspecies feed in lower-height young palms, they have not been reported thus far to attack tall coconut palms or to breed in the tops of dead poles (10). Adults were susceptible to *M. anisopliae* in New Britain (73).

Supplemental Material

Strategus aloeus

Strategus aloeus damages oil and coconut palms in South America (10, 27). An aggregation pheromone consisting of three components was identified (88, 90) (**Supplemental Table 2**) and associated with a "calling" behavior paralleling that of *Scapanes* species (88, 90).

MANAGEMENT BY *ORYCTES* NUDIVIRUS

OrNV is a key agent for *Oryctes* management in areas where it is not endemic, necessitating laboratory-based work since 1980, interconnected with fieldwork, to extend our knowledge of this pathogen.

History and Brief Overview

Huger (37) described his original discovery of the virus, and perspectives on its effects in different areas have been provided (29, 38, 39). Infection is peroral. The virus multiplies in the midgut and fat body of larvae and in the midgut of adults, which defecate the virus and thus are "flying virus factories" (37) responsible for its dispersal. Larvae die from the infection and their cadavers release new virus into the breeding sites, where adults are infected by ingesting it there or during mating. The adult life span is shortened and females cease oviposition. Safety testing showed it to be harmless to vertebrates and beneficial insects. The virus is readily propagated by infecting larvae but is rapidly inactivated in the environment (10, 106).

Classification and Structure

Although previously considered to be a baculovirus, OrNV is now classified as a member of the *Nudivirus* group (12, 100–103). The rod-shaped virion (37) is the infective unit in which OrNV must survive in the environment and is transmitted naturally and in artificial dosages. OrNV has a viral membrane and capsid enveloping a circular double-stranded DNA molecule, for which the sequence of 127.615 kb has been determined for an isolate (Ma07 = type B) from the midguts of adults from Malaysia (102). Twelve geographical isolates had small differences due to insertions or deletions (18). Antigen differences separate Philippines isolate PV505 from the isolate KI from Kerala, India (55). In maps of 96% of the OrNV genome of PV505 (14) and of KI from adults, differences in restriction endonuclease profiles were noted, and an estimate of the size of the KI genome of approximately 123 kb (56, 57) was close to that found later for the Malaysia isolate (102) of 127.6 kb. When three cloned strains were released on an atoll in the Maldives, after 4 years three genomic changes were detected in recovered isolates: a recombinant of two of the released strains, an insertion, and a point mutation (17). Morphogenesis and replication of OrNV have been followed in cell cultures of the dynastid *Heteronychus arator* (13, 16).

ELISA: enzyme-linked immunosorbent assay

Diagnosis and Detection Methods

An earlier method, which is still useful and convenient, used to detect hypertrophied nuclei of infected adult midgut cells (109) is the Giemsa stain smear technique, and it was applied in India (58), Oman (48), and Papua New Guinea (32, 91), while fresh mounts of squash preparations were used in Samoa (53). The Giemsa method was supplemented by ELISA (enzyme-linked immunosorbent assay) techniques (51, 55, 58, 77, 107) and then followed by PCR techniques for detecting OrNV DNA in midgut tissues from adults or larvae (80). ELISA and PCR can detect OrNV at an earlier stage of infection and in freshly dead adults. With all detection methods, it is essential that all specimens are kept isolated from one another from the moment of collection (84) to avoid cross-contamination giving unduly high incidences of OrNV. Where it is desirable to avoid sacrificing beetles, specimens may be kept individually and allowed to defecate into saline, which is then centrifuged and the sediment is resuspended for preparing Giemsa smears, with positive detections of infection appearing from day 3 postinfection (60). Alternatively, detection of OrNV in such cells by PCR might be applicable.

Inactivation

OrNV is rapidly inactivated when mixed and stored in larva-rearing medium (10). Inactivation has been assessed as the percentage of larvae that survive being fed OrNV subjected to a particular inactivating treatment. Infectivity is lost after 8 days if OrNV is mixed with cow dung at ambient temperature in India (54). This renders the virus vulnerable to dying out (106) and makes release of infected adults the preferred method of dissemination. Successful spread in the South Pacific suggests that transmission in populations outstrips the rate of inactivation (59).

The cause of inactivation remains unknown. Perhaps enzymes from bacteria or fungi present in the medium attack the proteins or DNA of the virion. Temperature inactivates the virus completely, by denaturing proteins or DNA, after being heated as a suspension at 56–70°C for 10 min (10, 59). There is little or no information about temperatures prevailing inside breeding or feeding sites in the South Pacific. Ground-up third-instar larvae midgut suspension was completely inactivated after 4.5 h at 37°C, and at a depth of 25 cm inside dung heaps in coconut palm areas in South India, summer temperatures varied between 30°C and 40°C but maxima did not last for a long time and thus were not expected to inactivate OrNV to adversely affect its survival and dissemination there (69).

Inocula, Virulence, and Resistance

OrNV for inoculum has been propagated in tissue culture of *H. arator* cells or prepared from guts of infected *O. rhinoceros* adults, and in sterile vials these preparations remain infective for two weeks to 3 months at 28°C, or for 20 weeks to one year at 4°C (15, 113, 115). Inoculum is applied to the mouthparts to infect beetles prior to their release. Differences in virulence of different geographical genomic isolates (18) have been suggested (110), although there are problems regarding dosages of virions administered (see Discussion, below). With this caveat in mind, an isolate from Leyte Island, Philippines, caused more larval mortality than did isolates from other locations in the country or from Samoa (110). In the Malay Peninsula, after standardizing doses following quantification by comparative PCR (although the number of virions in a dose is unknown), the isolate B (=Ma07), extracted from adult midguts from the west coast, caused higher mortality in larvae and adults and was deemed more virulent than the widespread isolate A (identical to isolate PV505 from the Philippines), and isolate C from Sabah caused the lowest mortality (84). Some evidence suggested *O. rhinoceros* adults from the Philippines were more resistant to isolates compared with adults from Samoa, whereas trials with larvae did not indicate resistance but suggested isolates differed in virulence (% mortality) (110). Possible host resistance was suggested in East Java and South Sulawesi (114).

Oryctes rhinoceros–OrNV–Palm Ecosystem

Where OrNV is already endemic, or once it is released and becomes established in nonendemic locations, a highly complex system of factors, variables, and interactions exists between palms, *O. rhinoceros*, and OrNV (**Figure 1**). Any patch of palms with these components present, from Mauritius in the west through Southeast Asia to Fiji and Samoa in the east, is a unique ecosystem with differing variables and interactions, which change over time.

Effects of Release Into Countries Where OrNV Is Not Endemic

Since 1980, OrNV has been introduced against *O. rhinoceros* into locations such as the Andaman Islands (India) (40), Minicoy Island (India) (61), the Maldives Islands (116, 117), and Oman (48), and damage subsequently fell significantly. A summary is presented in **Supplemental Table 3** (for contributors to this work up to 1980, see Reference 10). In view of the environmental complexity (**Figure 1**) and because releases were carried out in different ways and at different times and into different locations and subsequently monitored and measured by different methods, it is not possible to compare the effects of releases in detail. An overall feature is clear—in general, the higher the *O. rhinoceros* population and palm damage level are at the start of OrNV release, the better the conditions are for establishment and transmission and the more marked the subsequent reductions in damage (11) (**Supplemental Figures 12 and 13**).

Rerelease in Countries Where OrNV Has Established

In some countries where OrNV had been established, rerelease or a review of the initial release has been done.

Samoa. Work carried out in Samoa showed the problems that can be encountered in rerelease (53). The boundary between normal areas and outbreak locations targeted for OrNV rerelease can be subjective, particularly on large islands where coconut plantings extend for many kilometers, and each location incorporates the variables shown in **Figure 1**. The released mass-reared infected adults can disperse away from the outbreak areas and thus not contribute to the virus load in the population there. Virus incidence was monitored by removing considerable numbers of adults from the population with EC traps or from feeding holes in crowns, so it is not possible to separate this

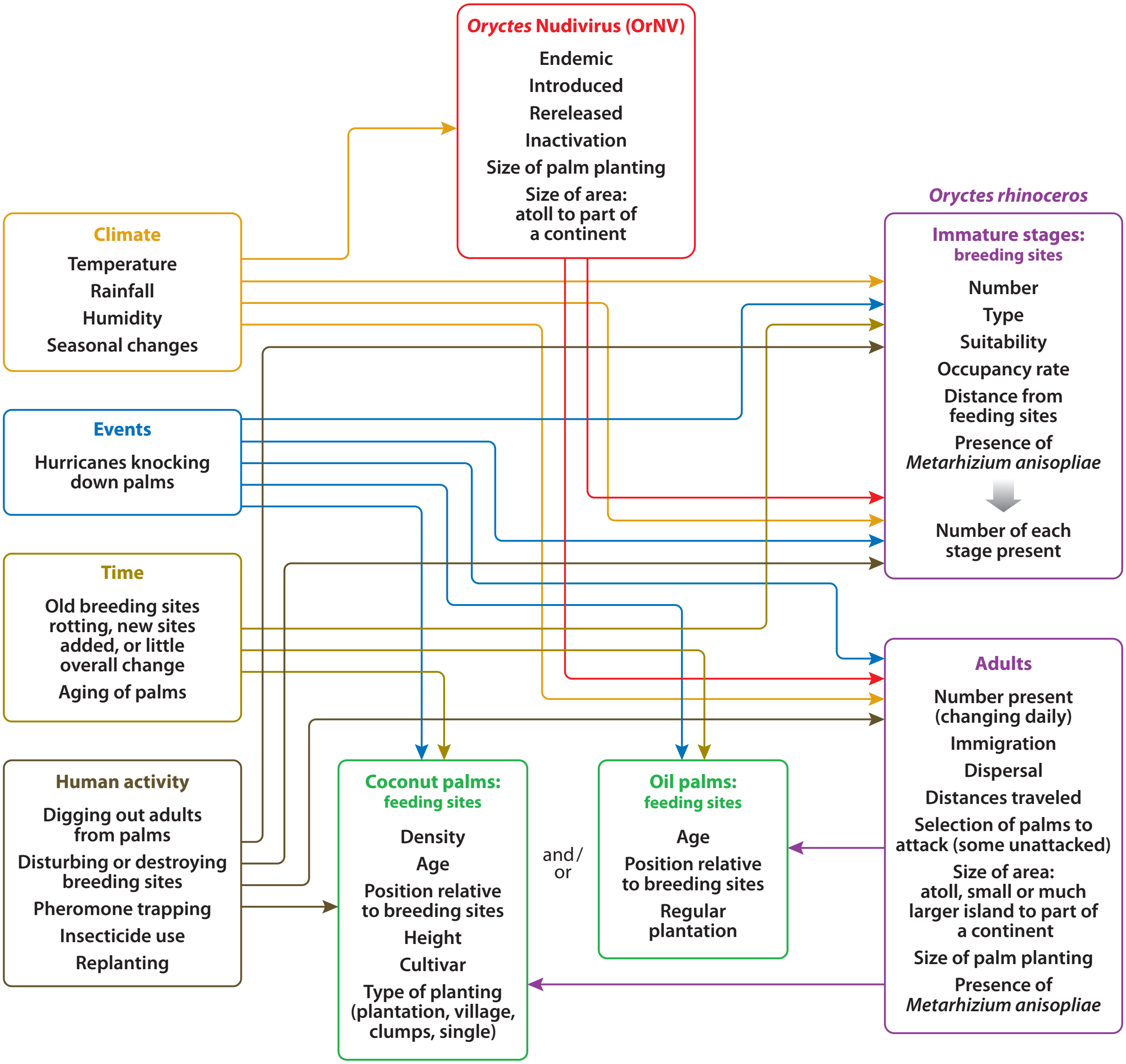

Figure 1

The *Oryctes rhinoceros*–*Oryctes* Nudivirus (OrNV)–palm ecosystem showing the key variables and their interactions. The variables and strength of interactions vary across the geographic range of the components.

effect on reducing the population from the effect of OrNV rerelease (53). The number of adults that were EC-trapped fell following releases (**Supplemental Table 3**) but could rebound. The proportion of males trapped fell.

Andaman Islands. After earlier suppression of *O. rhinoceros* by OrNV (40) (**Supplemental Table 3**), felling of coconut palms led to a resurgence, so adults dosed with a crude virus preparation from larval midguts, which gave optimum longevity and reduced fecundity,

Supplemental Material

were released on South and Little Andaman Islands, India, and damage fell markedly (71, 72) (**Supplemental Table 3**). Booster release was suggested when damage to fronds or spindles exceeded 10% (72). These reductions in the *O. rhinoceros* population may have been aided by the relatively small size of these rerelease islands, which confined the effect of the OrNV.

Tonga. When Tongatapu was resurveyed in 1978 (107), seven years after initial release and with no further releases, over 84% of adults were infected as determined by ELISA (**Supplemental Table 3**). There was no recommendation for further releases at that time.

Effects of Further Release in Countries Where OrNV Was Endemic

Releases have been tested in countries where OrNV is endemic.

India. When OrNV-infected adults were released in Kerala, the number of oil palms with central spear damage fell from 71% to 21% (23), and coconut fronds damaged over 3 years fell from 34% to 7% (6).

Malaysia. In Malaysia, the incidence of OrNV varied widely. Thirty-three to 65% of pheromone-trapped adults and zero to 52% of adults from breeding sites were infected (84). At an oil palm location on the west coast, 38% of trapped adults were infected with isolate A. Adults were infected with the more-virulent isolate B and released, and small samples of the natural population were tested. Eleven months later, more than 90% of trapped adults were infected, and isolate B dominated, having spread from the release site and apparently outcompeted isolate A, whereas 5 months after release, 11% of larvae were infected, but this level soon fell. This low incidence in immatures suggested little transmission occurred among them, so at locations where breeding sites were abundant, there was a high probability of many new healthy adults emerging to boost damage, even when the incidence of OrNV among adults was relatively high. This finding suggested adults were becoming infected after first emergence from their breeding sites (84).

Philippines. On southeast Luzon, in a location where dead poles were scarce and ground trunks abundant, a release of OrNV-infected adults and later of laboratory-reared healthy adults increased the incidence of disease among young wild adults feeding in palms, with the infection level peaking four months after both releases, perhaps due to the increased population density boosting transmission. After both releases there was no rise in incidence of wild beetles caught in EC traps, i.e., beetles seeking breeding sites (112). Although these results were considered to be due to transmission occurring in live palms, as copulations have not been observed there (see Discussion, below), transmission may have occurred during earlier visits to breeding sites. As the percentage of infections rose, the proportion of trapped males fell, as in Samoa (53).

Model for effect of OrNV release and establishment. Hochberg & Waage (36) postulated that transmission between young feeding adults was the key pathway, and after OrNV release in a nonendemic location, an epidemic wave would settle into an equilibrium within 2 years, with incidence of disease approximately 29–54% in adults and 3% in larvae and immature adults. A further model incorporating host density-dependent reproduction and dispersal raised the possibility of cycles of outbreaks occurring in adjacent areas simultaneously, followed by waves of OrNV reappearing regularly (24).

OrNV Release Against *Oryctes monoceros*

After OrNV release, damage fell but stayed the same in nonrelease control sites in Tanzania (75). In a later study (92), damage fell and then fluctuated, and this also occurred in control areas, but it is possible that OrNV had spread to control areas. The dry conditions may have inactivated the virus, and low larval mortality meant little OrNV contamination of breeding sites and thus reduced transmission to visiting adults, so alone OrNV could not exert sufficient control (**Supplemental Table 3**). Manually extracting adults from young palms and destroying breeding sites, concurrently with OrNV, were recommended. Trapping and collecting from breeding sites in and around study areas disrupted populations being observed, so a suggested OrNV release on small islands with high initial levels of damage may give a better indication of the effect of OrNV (92). In the Seychelles archipelago, OrNV became established between 10 and 16 weeks (49) (**Supplemental Table 3**).

OrNV and *Strategus aloeus* and *Scapanes australis* Subspecies

Adults of *S. aloeus* supported extensive OrNV replication in the midgut, so the pathogen may have potential for biological control of this species (50). Whereas OrNV caused mortality in *Scapanes australis grossepunctatus* larvae (10), only limited replication occurred in adults and none occurred in *S. a. salomonensis* adults (50), suggesting that OrNV had little potential for controlling *S. australis*.

MANAGEMENT BY PHEROMONE TRAPPING

The discovery of E4-MO as the male-produced aggregation pheromone of *O. rhinoceros* and *O. monoceros* and its commercial synthesis (64, 76) led to E4-MO rapidly superseding the earlier synthetic attractant, EC. Trapping with E4-MO as a management component is now applied in Malaysia and Indonesia (65, 67) and is used as a tool in ecological studies. Several trap designs of differing cost and effectiveness using E4-MO emitted from a sachet dispenser have been tested (22) (though not all trap types have been tested simultaneously at the same location):

- Plastic bucket trap: plastic bucket placed on a support, or partly sunk into the soil (pitfall), with the concave cover upside down and perforated with five beetle-sized holes (67);
- Parabolic trap: a zinc dish inserted into a plastic drink bottle where the bottom has been cut off;
- Barrier, i.e., single- or double-vane, traps: one or two metal vanes with the pheromone dispenser in the center, standing in a funnel inserted into a plastic bucket (pail) (illustrated in Reference 67); and
- PVC tube trap: a tube 16 cm in diameter and 2 m high with two side openings or windows in the upper part and an open top, which stands in a bucket and simulates an upright coconut trunk. It catches more beetles than the bucket trap, and adding empty oil palm fruit stalk material enhances the catch (63).

Oryctes rhinoceros

Research to develop the use of pheromone trapping as a management tool against *O. rhinoceros* has been conducted in southeast Asian countries (67) such as India (41), Indonesia (22), and Malaysia (65).

Malaysia. With one trap per 2 ha (**Supplemental Table 2**), infestation was maintained below 10 individuals (mainly larvae) per square meter of oil palm breeding site, until breeding ceased after

24 months (45). Without traps, immature stages soon built up and persisted after 26 months, though at one stage they suffered a bout of high mortality due to pathogens, a possible density-dependent effect.

IPM: integrated pest management

Traps were used to monitor immigration into a 4.5-ha block beginning 5 months after replanting (65). Infestation of the breeding sites in the center of the block occurred 4 to 7 months after they were chipped. Females moving in from adjacent mature plantings were trapped more at the edges than in the center and this correlated with the number of second-instar larvae later found in the breeding heaps. Trap catches showed an increase in flight activity during wet weather and by males during a full moon. Sometimes *O. rhinoceros* is attracted to light traps, and screening polymorphic loci of the genome of populations from four locations in Malaysia and Indonesia suggested the possible occurrence of different cryptic (i.e., not morphologically distinguishable) species attracted only to E4-MO traps, or only to light traps, or to both (52). In Malaysia, a double-vane trap has been adapted and tested to attract beetles, automatically infect them with *M. anisopliae*, and release them to disseminate the pathogen into shredded oil palm trunk breeding sites (83).

India. A trial using bucket traps in coconut plantations (**Supplemental Table 2**) caught a high percentage of unmated females, so mass-trapping is considered beneficial in reducing beetle populations (41). Another study in an oil palm plantation using 1 trap per 2 ha resulted in a considerable reduction in damage; thus, traps could well be incorporated into an integrated pest management (IPM) program (70). A trial using double-vane traps in an oil palm plantation in Andhra Pradesh (44) found that the pheromone evaporated quickly on days when temperatures were above 33.5°C, reducing its longevity, and some adults attracted to the traps were not caught and could attack nearby palms. In this case the traps were not considered economical. These results suggested that the economic advantage of trapping could vary with location.

Supplemental Material

Oryctes monoceros

Although trapping in the Ivory Coast helped remove gravid females (**Supplemental Table 2**), 62% of all beetles found in feeding holes in palms were females, of which 46% were young and immature and 24% unmated, or had just laid eggs and were ready to feed again (4). Both sexes usually occurred alone, and these feeding beetles were unresponsive to traps and caused potentially lethal damage to young coconut palms. There are no data on physiological changes that affect male behavior, but Allou et al. (4) suggested that, when ready, males station themselves in breeding sites and emit pheromone to promote mating there.

***Oryctes elegans* and *Scapanes a. grossepunctatus*.** Trials with pheromone traps caught considerable numbers of beetles and indicated that trapping may have potential against these species (86, 87). Details are available in **Supplemental Table 2**.

DISCUSSION

OrNV

Despite the variability in *O. rhinoceros*–palm ecosystems, up to and since 1980 introducing OrNV into locations where it is not endemic has led to lower beetle populations and reduced damage (**Supplemental Table 3**). In the countries of the South Pacific and Indian Ocean where OrNV had been introduced prior to 1980 and had lowered populations, although mentioned at times in government annual reports (e.g., Fiji), there have been no further peer-reviewed publications on its effects in the decades since 1976 for Fiji, 1980 for Papua New Guinea, 1982 for Samoa, 1981 for Tonga, and 1978 for Mauritius.

The possibility had been raised (10, 38, 39) that if an unusually large number of breeding sites are created locally, e.g., by palm felling or cyclones (**Supplemental Figure 5**), the *O. rhinoceros* population could resurge in these South Pacific coconut palm "outbreak" areas and increase damage despite the presence of OrNV (10). However, to test this would require researchers to survey damage regularly, to provide a baseline from which changes could be measured subsequently, and to provide an objective criterion for recognizing when the observer had passed from an area of "normal" background damage (which occurs wherever *O. rhinoceros* exists) into an area of "outbreak" damage.

In countries where OrNV is endemic, e.g., Malaysia, the virus is not relied on as the chief control agent in oil palm replanting areas where large amounts of shredded old trunk material temporarily provide abundant breeding sites and allow an increase in the *O. rhinoceros* population, despite a high incidence of OrNV among the adults (84). Hence, the use of pheromone traps and the application of *M. anisopliae* to the breeding sites, as components of IPM, have garnered much interest. Hochberg & Waage (36) suggested that boosting the incidence of *M. anisopliae* by utilizing it as a biopesticide against larvae may reduce emergence of adults acting as "flying virus factories," to the extent that OrNV might die out in certain locations. Although perhaps possible on small islands, on larger islands or continental landmasses, it seems likely that infected adults flying in from outside the treated areas would replenish OrNV. In a commercial oil palm setting, any reduction by *M. anisopliae* in immature stages or older adults visiting breeding sites would likely lead to a beneficial reduction in adults attacking palms at that location, unless there was substantial immigration from outside.

For areas where OrNV is not endemic, this virus is the only significant biological control agent against *O. rhinoceros* to emerge in over 40 years of research, and on the basis of accumulated knowledge, introducing or boosting the level of OrNV at a location is best done by releasing infected adults. Adults can be obtained either by pheromone trapping of the wild population or by mass-rearing, and both are expensive in terms of equipment, facilities, servicing, and labor. It is uncertain how long adults can survive at the temperature inside traps, and there is no way of determining whether trapped adults are infected or healthy, except by holding each beetle individually and testing its excreta (e.g., 60) (or by PCR), which takes time and technical expertise. Infection also shortens the life span of captured adults. If all trapped adults were simply reinfected (2, 42), this could include a high percentage that were already infected, and there is no information available on the physiological effect or economic advantage of reinfecting and releasing already infected adults. It might even be suggested that infected adults were better not trapped at all, but left free to act as vectors (e.g., as used in 84). Also in large-scale commercial plantings with an investment in traps, there could be reluctance to release trapped adults.

There are suggestions (e.g., 72) but no definite coconut palm damage level at which re-release of infected beetles would be considered or advisable at a location, nor what a suitable number to release would be (**Supplemental Table 3**). On large islands with extensive palm plantings, the effect of rereleases can be difficult to gauge because an unknown proportion of adults can disperse from the area being observed (53), and the effect could well be different at each location at the time [e.g., in the Philippines where rerelease was proposed (2, 42) to be carried out by farmers or extension workers inoculating adult *O. rhinoceros*] (considering the variables shown in **Figure 1**). The effect of rerelease might be more noticeable on small islets (92). In a coconut palm area, the expensively obtained, to-be-released OrNV-infected adults need to be kept free of *M. anisopliae* because if they die of this fungus, their usefulness as OrNV vectors is nullified.

Hochberg & Waage (36) have proposed a model in which OrNV is transmitted from feeding adult to feeding adult, occurring predominantly in palm crowns. Copulation has been observed in split-log breeding site traps (19), and similar proportions of both sexes were found together (but not in the same feeding hole) in crowns (33, 108) and decaying logs (108, 112). However, mating by *O. rhinoceros* in crowns or at the entry to feeding holes has not been observed. This question of OrNV transmission ties in to that of where pheromone is released to promote mating (see below). Although it is plausible that infected adults may defecate OrNV at or in their feeding holes, no published report has indicated OrNV has been found there, or how long the virus would persist, or the likelihood of another adult visiting the same hole and becoming infected, before OrNV becomes inactivated and disappears.

Although "in general, the greater the geographical separation of (OrNV) isolates, the greater the genotypic variation" (18), even within the same area of Luzon, Philippines, five isolates were found. Moreover, isolate A on the Malaysian Peninsula is identical to PV505 (84) from Luzon, approximately 2,300 km away across the South China Sea, with the only land connections, interrupted by stretches of ocean, being to the north along the coastal Asian mainland or to the south via Sabah where isolate C is now found.

Problems arise in discussion of earlier studies on virulence of geographic isolates as the doses and resulting LD_{50} are expressed as amounts of suspended OrNV-infected tissue (ground-up infected larvae fed to larvae or hemolymph from infected larvae injected into adults), so the number of infective virions in doses is always unknown (10). Also, the meaning of "virulence" could vary among authors. Although such dosages can be sufficient for field releases (71, 72), exploring "virulence" in the laboratory requires accurate doses in number of virions per milliliter, as has been advocated (39), though there may be difficulty in achieving this in practice. A solution might be quantitative estimation of the amount of a given DNA sequence present in samples by comparative PCR (84), which may be a reproducible index of the number of virions in a dose. Another problem with the LD_{50} measure is that it means 50% of a test batch of larvae or adults receiving the dose (presumably a large number of virions) survive—a conundrum for which there is currently no explanation. In the field, virion doses of any ingested isolate would be unknown. The result of a higher dose of a less-virulent isolate might be equivalent to that of a lesser dose of a more-virulent isolate. From an ecological standpoint, if virulence is defined as causing more rapid kill, it has been noted (105) that selecting more-virulent strains for release, leading to earlier death, could be detrimental to the transmission of OrNV, as it would result in more rapid death of larvae, with subsequent decomposition and inactivation in breeding sites, and a shorter life span for adults during which they could act as mobile virus factories for OrNV dissemination. For this reason, less-virulent strains may evolve and come to be more widespread in the wild (2; and perhaps isolate A, see Reference 84).

OrNV "appears to be endemic" in Sri Lanka (42), but it is difficult to find references to its occurrence there, so it would be of interest to confirm its incidence and effects in view of its close proximity to Kerala, south India. Knowing whether other dynastids in plantations in Southeast Asia or hitherto untested *Oryctes* spp. elsewhere, are susceptible to OrNV (21) would be useful, as they might be potential reservoirs or targets for the pathogen. Now that *O. rhinoceros* has invaded well-separated islands in the west South Pacific and Indian Oceans and has been established there for many decades, has this geographic and reproductive isolation begun to cause local differentiation in the genome of these populations, perhaps affecting their susceptibility to OrNV?

Pheromones

Although the aggregation pheromone of *O. rhinoceros* attracts adults to traps (35, 62), it would be of interest to know where males in the wild release it. Is it released in the crown, in frond axils, or at the entry to feeding holes, where mating has thus far not been directly observed? Is it released only at breeding sites? Is it released at both breeding and feeding sites? Conversely, does *S. a. grossepunctatus*, which releases pheromones at the entry to feeding holes to promote mating also release pheromones at breeding sites? Why do the species studied thus far emit aggregation pheromones, attracting other males as well as females. In *S. a. grossepunctatus* this results in competition for a female, even combat between arriving males and the emitting male that had started the hole (74). Is the purpose sexual selection and fitness? *S. a. grossepunctatus* couples could regularly occur together in a feeding hole, with the female deepest in the hole (10), so after mating the female must have pushed on past the male. *O. rhinoceros* was usually found singly in holes (10, 33) but more than one individual may attack a palm at the same time, whereas neighboring palms remain unattacked (33). Because the body of the male is positioned at a right angle to the body of the female during copulation (33), there would seem to be insufficient room inside the feeding hole for the copulating position. Is a calling behavior involved, as in *Scapanes* spp.?

The considerable investment in and commitment to pheromone trap deployment and servicing as a component of IPM against *O. rhinoceros* appear to be accepted by oil palm plantations, taking into account their organization, the value of the crop, the eventual growth of the palms past the most vulnerable young stage, and the eventual rotting away of the breeding sites. Their use in coconut palm growing areas could be more problematic and affected by factors such as the varying size, age, and accessibility of plantings, use to be made of the crop or revenue produced, government funding, and socioeconomic factors. In either situation it may be difficult to estimate what proportion of the wild population originating in, or immigrating to, the trap deployment area is caught by the traps. Because every location is a different ecosystem with its own variables (**Figure 1**), the effectiveness of traps as management tools may well change with population size and catch only a portion of the component of the population that is in a physiological condition to go to traps at that time, as found for *O. monoceros* (4). Traps baited with E4-MO are competing with emitting wild males. Anatomical and histological studies of the glandular tissue producing aggregation pheromones, as well as biochemical studies of the enzymatic pathways that produce these compounds from components originating in ingested palm sap, would be of interest.

More data would be opportune on the extent to which *O. agamemnon* adults damage the date palm, where mating occurs, whether an aggregation pheromone is produced, and whether adults are responsive to known dynastid pheromones. The aggregation pheromone of the date fruit stalk borer *O. elegans*, 4-methyloctanoic acid, is only weakly attractive alone but synergistic with fresh date palm tissue odor (86), perhaps coming from cut surfaces. In the wild, date, coconut, or oil palms might emit particular volatiles or odors from beetle feeding holes or any other damaged tissue, or even from undamaged palms, that attract rhinoceros beetles to palms and not other trees. Perhaps these volatiles later act together with the male aggregation pheromone, of which a number of components are small common molecules (88). Conversely, might a palm, wounded by beetle attack, in response produce defensive phytochemicals aimed at warding off further attacks?

M. anisopliae could be tested against *Oryctes* spp. attacking date palms. In coconut palm growing areas where the fungus is endemic, it might help control *O. rhinoceros* larvae in friable material (compost, sawdust, or cow-dung heaps), but determining to what extent *M. anisopliae* is able to penetrate decaying logs or stumps to reach *Oryctes* stages, or to disperse away to boost the background level in the area, is problematic.

Detailed and rapid damage surveys are still the most convenient index for monitoring on an ongoing basis *O. rhinoceros* populations in coconut palms of all heights, and they avoid disrupting the ecosystem in study areas because they do not require the removal of larvae or adults. In coconut plantations, sanitation (i.e., the removal of fallen trunks), if practiced, continues to make an important contribution to *Oryctes* management. There are at least 13 species of *Oryctes* in Madagascar and the Comoros Archipelago, and their biology remains unexplored (10).

SUMMARY POINTS

1. The range of breeding sites utilized by *O. rhinoceros* has been extended, underlining its versatility.
2. The entomopathogenic fungus *M. anisopliae* has yielded promising results as a biopesticide against immature stages of *O. rhinoceros* in friable breeding sites, such as chipped oil palm trunk material, decaying coconut palm fronds, and cow-dung heaps.
3. Significant reduction in *O. rhinoceros* populations and damage to coconut palms has followed releases of OrNV using beetles as carriers into new locations where the pathogen was not present.
4. Data on molecular biology and ultrastructure of OrNV have been gathered, but discussion of virulence of different strains or isolates has been clouded by past inability to measure administered doses of virions. Quantitative PCR of DNA segments might be further developed to obtain a reproducible index of virion concentration in doses, essential for comparison of virulence, however defined, of different isolates. Transmissibility of an isolate might be a more important aspect of virulence than time taken to kill, or percent mortality, given the likelihood of inactivation.
5. More observational evidence is needed to determine whether mating of *O. rhinoceros* occurs in palm crowns or whether concomitant transmission of OrNV might occur at this possible mating site.
6. Male-produced aggregation pheromones have been identified in several palm-pest dynastids, have been developed for use in IPM on a commercial scale against *O. rhinoceros*, and have potential to be used against other species.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I thank Dr. R.W. Baker, School of Chemistry, University of Sydney, for drawing the pheromone molecules, and Prof. L. Hughes and Prof. M. Westoby, Department of Biological Sciences, Macquarie University, for advice.

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