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Coconut rhinoceros beetle, *Oryctes rhinoceros*: A manual for management of the pest in Pacific Island countries and territories

Second edition



Coconut rhinoceros beetle, *Oryctes rhinoceros*: A manual for management of the pest in Pacific Island countries and territories

Second edition

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Pacific Community
Communauté du Pacifique

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Introduction to the second edition

This manual has been developed to support trainers in building the capacity of biosecurity and extension workers for the management of coconut rhinoceros beetle (CRB) in the Pacific region. It draws on extensive literature on CRB, especially as it relates to the Pacific Island countries and territories (PICTs), as well as knowledge from current and former colleagues who have worked to manage this pest.

Publication of the first edition in 2020 was timely, as the Pacific region is being challenged by the invasion of a new CRB haplotype, CRB-G (Clade I; Marshall et al. 2017), and there remains a need to regain control of the long-established haplotype CRB-S (Clades II and III), which has hampered the success of renovation programmes for mature tall palms as well as newly emergent, high-value coconut product industries (e.g. virgin oil and coconut water) that offer economic opportunities for villagers in the region. Unmanaged CRB populations continue to threaten Pacific livelihoods. This second edition is an opportunity to update recommendations for CRB control.

This manual is aimed at the new generation of scientists, technicians and extension officers who are tasked with controlling invasive species and promoting local agricultural initiatives. It is not intended to replace or substitute reviews from Bedford (1980, 2013), Huger (2005), Godshen (2015), or the numerous works of Zelazny and others, from which this manual draws extensively. Summaries on CRB are also provided by CABI (2015) and PESTNET (2021). Key documents are available from the Pacific Community (SPC) and a thorough and extensive list of scientific papers pertaining to CRB can be found in Godshen (2015). Information provided in this manual is to support trainers and leaders to understand CRB biology, identify CRB invasions, and take appropriate action in order to reduce the impact caused by the invasions.

This second edition is divided into four sections. The first section provides a brief review of CRB in the Pacific and an update on the status of the pest in the region. The second section provides information to support recognition of the pest and its associated damage, as well as methods for collection and handling of the pest after it has been recognised. Relevant contacts are also provided to facilitate access to expert assistance, where needed, and methods are outlined for further diagnostics. The third section builds on this knowledge to describe how to: (a) conduct delimitation surveys; (b) measure the severity of palm damage; (c) monitor CRB populations using pheromone traps; and (d) provide a baseline to monitor the effectiveness of control actions. The fourth section covers control actions for CRB in the Pacific and draws on the experiences of our colleagues, particularly those working in Guam, Solomon Islands, Papua New Guinea and Vanuatu, to control 21st century CRB invasions. These control strategies are brought together into an integrated pest management (IPM) plan for CRB.

The re-emergence of CRB as an invasive pest in the Pacific underpins the need to review and revitalise the IPM system for CRB, to protect coconut and oil palm production. Since the first edition was published in 2020, there have been substantial gains in our understanding of these recent invasions. This second edition brings together this new knowledge in one place to support those on the frontline of CRB management.



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Photo: Iriarte et al 2015

1 Coconut rhinoceros beetle in the Pacific

1.1 Review

For over a century, Pacific Island countries and territories (PICTs) and their authorities have been confronted by an invasive insect from Asia, coconut rhinoceros beetle (CRB; *Oryctes rhinoceros* L.). CRB was inadvertently introduced in 1909 into Upolu, Samoa, inside pots containing rubber seedlings imported from Ceylon (now Sri Lanka), where it became established on the island, multiplied and spread rapidly, causing extensive damage to coconut palms (*Cocos nucifera*) and requiring measures for its management. The initial invasion, reviewed in Bedford (1980), spread throughout Samoa and American Samoa as well as Niuatoputapu Island (Tonga) (where it was eradicated) and onward to Wallis Island, Tongatapu (Tonga), Tokelau Islands and Fiji, where it had moved to most of the inhabited islands by 1953. Interestingly, DNA analysis of specimens from the main islands indicates that they belong to the same haplotype, Clade III (Marshall et al. 2017), suggesting that the population expansion was indeed caused by the original introduction of CRB to Samoa in 1909.

A second invasion of the Pacific appears to have started with shipping and material movements during the Second World War (Figure 1.1; Paudel et al. 2021). By the 1960s, CRB was established on the outer islands of Papua New Guinea (PNG), including Pak, Manus, New Ireland and New Britain, and on the islands of Palau (Gressitt 1953; Bedford 1980). DNA analysis identifies these CRB populations as haplotype Clade II, distinguishing them from the original invasion of Samoa (Marshall et al. 2017).

The invading populations caused extensive damage to coconut palms, which were the "tree of life" for Pacific peoples and had been in plantations for copra production since

the late 19th century. On Samoa, communities were mobilised to collect CRB and clean breeding sites. However, only a major ongoing effort, including legislation and fines, was able to maintain the beetle at tolerable levels. CRB had a similar effect on other islands, where controls were not attempted or were ineffective; losses of 50% or more of the standing palms were reported in some areas (Gressitt 1953). Scientists recognised that the CRB invasion around the PICTs was much worse than in South and Southeast Asia and speculated that natural predators were lacking in locations where the pest was most invasive. This phenomenon – in which a pest upsurge is noted after establishment in a new area without natural enemies – has since been described as "natural enemy release" (Elton 1958).

Recognising the importance of the problem, international agencies, including the United Nations Development Programme (UNDP), the UN Food and Agriculture Organization (FAO), and the Pacific Community (SPC, known at that time as the South Pacific Commission), funded a series of projects from the 1960s to the 1980s to develop control methods for CRB (Young 1986). These included pheromone trapping, biological control and integrated pest management (IPM). Many of the discoveries and methods developed during this era are still in use today.

1.2 CRB biological control: historic successes and new challenges

Although efforts were made to identify putative natural enemies, it was not until the 1960s that Dr Alois Huger discovered a virus in Malaysia with real potential to control CRB in the Pacific. The virus was introduced into Samoa where it rapidly spread and, subsequently, was redistributed to the other infested islands where it infected the beetle populations, spread, and reduced palm damage (Bedford 1976). The history of the virus discovery and its use are described by Huger (2005) and Jackson (2009). This virus was recently renamed *Alphanudivirus oryrhinocerotis*, however Oryctes rhinoceros nudivirus, usually abbreviated to OrNV, remains the common name (van Oers et al. 2023). The virus has been characterised by whole genome sequencing (Wang et al. 2008). In recent years, use of DNA PCR detection methods, supported by histological examination of gut tissues, have shown that OrNV has persisted in beetle populations where it was originally released (Marshall et al. 2017), and that the reduction in palm damage first noted following the initial virus release has been maintained (Bedford 2013).

The OrNV release programme was so successful that no further spread of CRB from the virus-infected populations was reported. However, while the virus weakened the infected beetle populations and reduced damage, it was not the complete solution. Beetle attack could still be highly damaging in areas with a large amount of breeding material, such as felled palms after cyclones or replanting, or unmanaged heaps of organic matter. For this reason, and to avoid excessive damage, IPM – including the use of biological, chemical and cultural controls – has been promoted for CRB in the Pacific Islands.

In 2007, a new and highly damaging outbreak of CRB was reported from the island of Guam. This marked the start of a new 21st century wave of damaging CRB invasions reported from PNG (Port Moresby) in 2009, Hawai'i (Honolulu) in 2013, and Solomon Islands (Honiara) in 2015. Genetic analysis of the mitochondrial *COI* gene defined the new invaders as belonging to a distinct haplotype of the beetle, CRB Clade I (also known as CRB-G), which could be differentiated from the historic CRB populations that invaded the Pacific during the 20th century (CRB Clades II and III, also known as CRB-S; Marshall et al. 2017). Invasive populations of CRB Clade I have arrived free from OrNV and bioassays have produced little or no infection by the OrNV isolates traditionally used to control CRB (Clades II and III) in the Pacific.

Population genomic analysis suggests that the new 21st century wave of CRB invasions has been mediated through human transport from a source in the Asian Northwest Pacific (Reil et al. 2018). CRB has continued to spread to new locations around the Pacific, including Rota in 2017, Vanuatu and New Caledonia in 2019, Marshall Islands in 2023 and Timor Leste in 2024 (Figure 1.1; Paudel et al. 2023, 2025). Confirmation of CRB presence in Mexico during 2024 is the first record of this pest from the Americas (Jackson et al. 2025). These new invasive populations continue to arrive free of OrNV infection.

Management of new invasions and improving biosecurity measures to limit further spread are significant challenges for the PICTs. In 2022, the first Pacific Ecological Security Conference included CRB as a central topic and produced a strategic action plan for the region (Hoffmann et al. 2024). The action plan recognised that the PICTs can be placed into three groups: (1) those without CRB; (2) those that have managed CRB populations after invasion during the 20th century; and (3) those that have been invaded by CRB during the 21st century. PICTs without CRB need assistance to improve surveillance and prepare response plans should CRB be detected, so that they can act quickly. PICTs with established CRB populations need support to ensure CRB is managed effectively, and the risk of further spread is reduced.

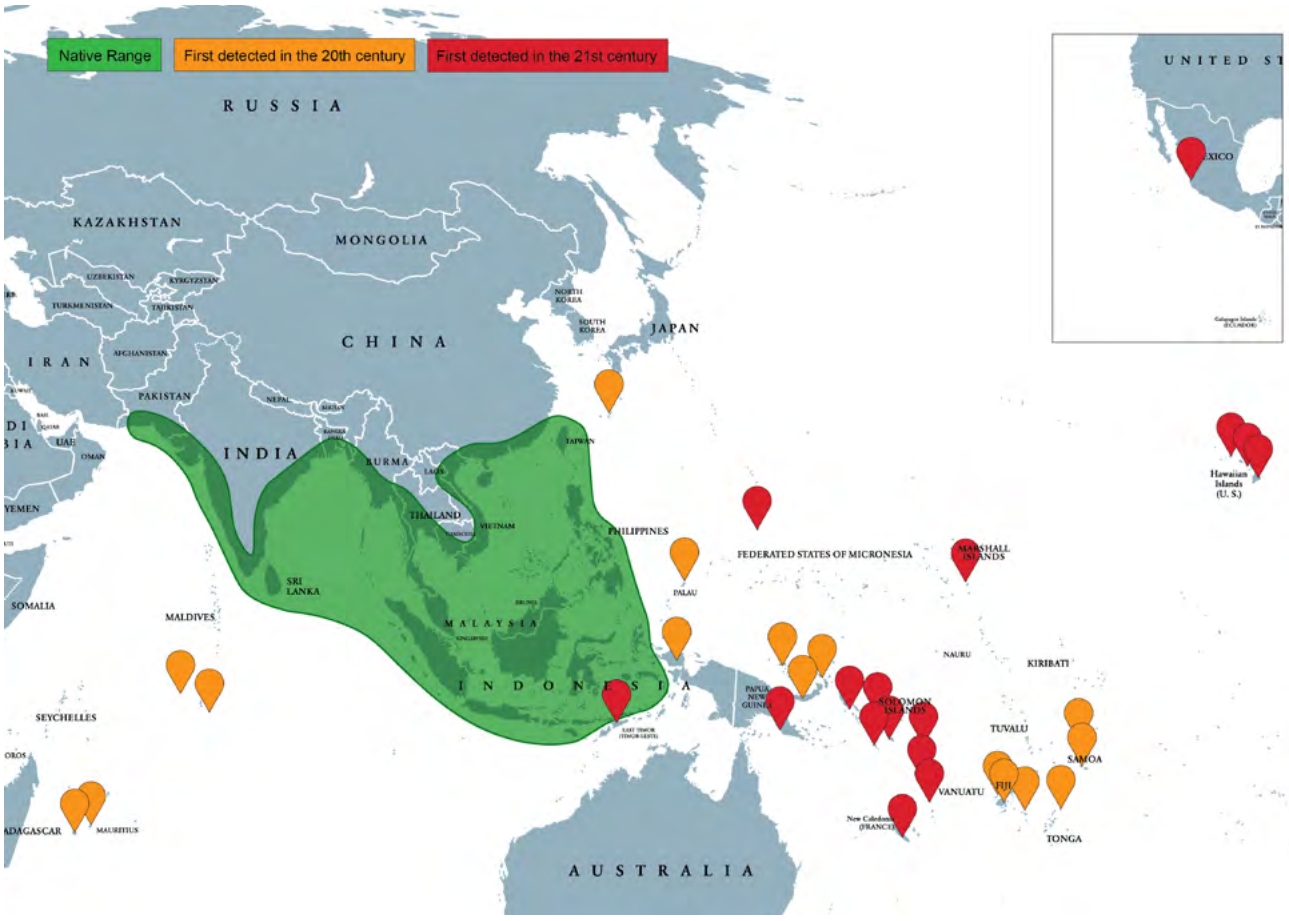


Figure 1.1 Geographic distribution of CRB including its native range (green shading), historic 20th century invasions (orange markers) and the new wave of invasions during the 21st century (red markers).

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2 Pest and symptom recognition

2.1 Beetle life cycle and recognition

CRB belongs to the subfamily Dynastinae of the large Scarabaeidae family of beetles. The Dynastinae include a wide range of large, horned beetles with many species endemic to Asia. Natural diversity decreases with distance from the Asian mainland with no endemic species from this group present on the smaller islands of the central Pacific. CRB has the typical scarab beetle life cycle, growing from egg to adult through three larval stages (instars) before transforming through pupation into an adult (Figure 2.1).

The duration of the life cycle depends on environmental conditions, but the time from egg to adult can be as little as 5 months in conditions with adequate food, temperature and humidity. The female beetle lays eggs after mating in rotting palm trunks or other organic matter (palm debris, compost). The eggs are laid individually – approximately three per beetle per week, resulting in an estimated 100 eggs per female. The eggs are white, elliptical (3.5 × 4.0 mm), and produce the first instar larvae in 8–12 days. Larvae are C-shaped, whitish with a reddish-brown head capsule with sclerotised mouthparts, and a darkened area visible in the posterior abdomen, known as the fermentation chamber, indicating the internal modification of the gut (Figure 2.2).

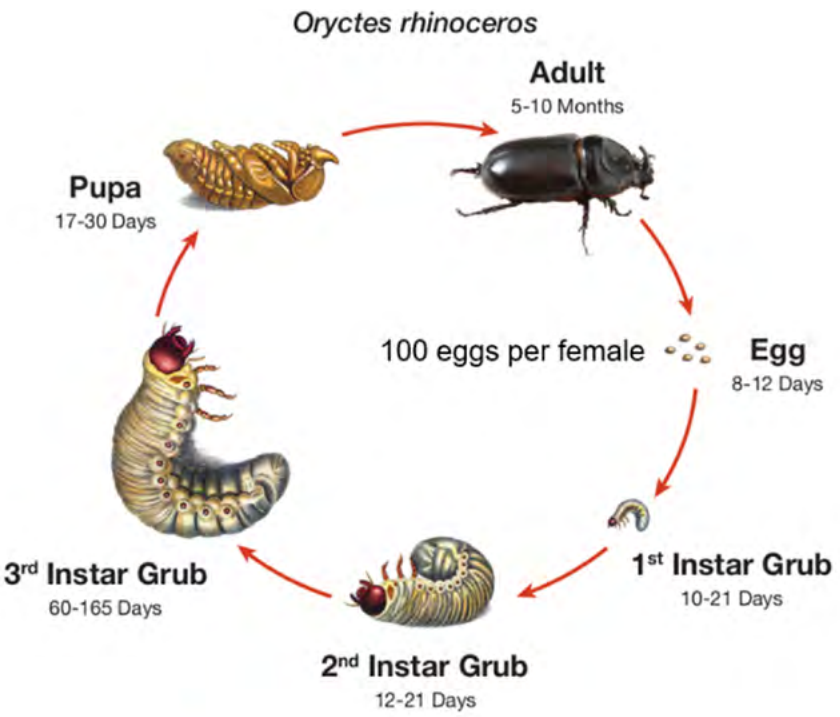


Figure 2.1 Life cycle of the CRB (University of Guam).

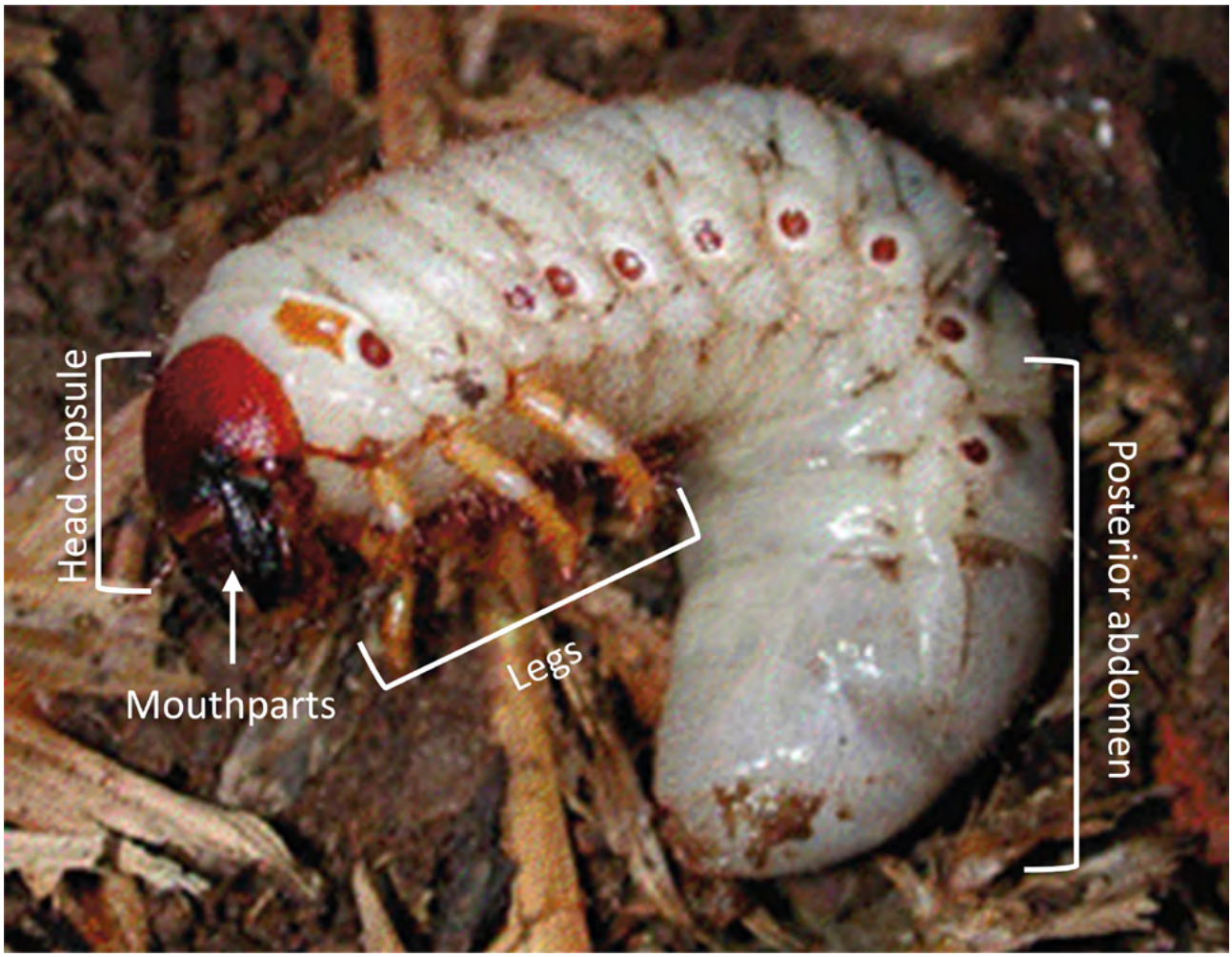


Figure 2.2 Third instar CRB larva. The fermentation chamber (part of the digestive system) lies in the posterior abdomen.

The larvae grow rapidly, passing through each of the first and second instars in approximately 2–3 weeks, before entering the longer third instar stage (9–24 weeks) where the larvae accumulate most of their mass, growing up to 100 mm in length and about 10 g in weight (Figure 2.2). Prior to pupation, the larvae stop feeding and their bodies change to a creamy colour and shrink slightly. The prepupal larva forms a cell and differentiates into the pupal stage, which lasts for approximately 3 weeks before metamorphosing into the adult beetle.

The adult beetle emerges from the puparium and remains in the feeding site for several days while the chitinous exoskeleton hardens. The beetle is large (30–60 mm in length and 4–12 g in weight depending on growth conditions), dark red-brown to black in colour, with the head, pronotum and elytra (wing cases) observable from above (Figure 2.3).

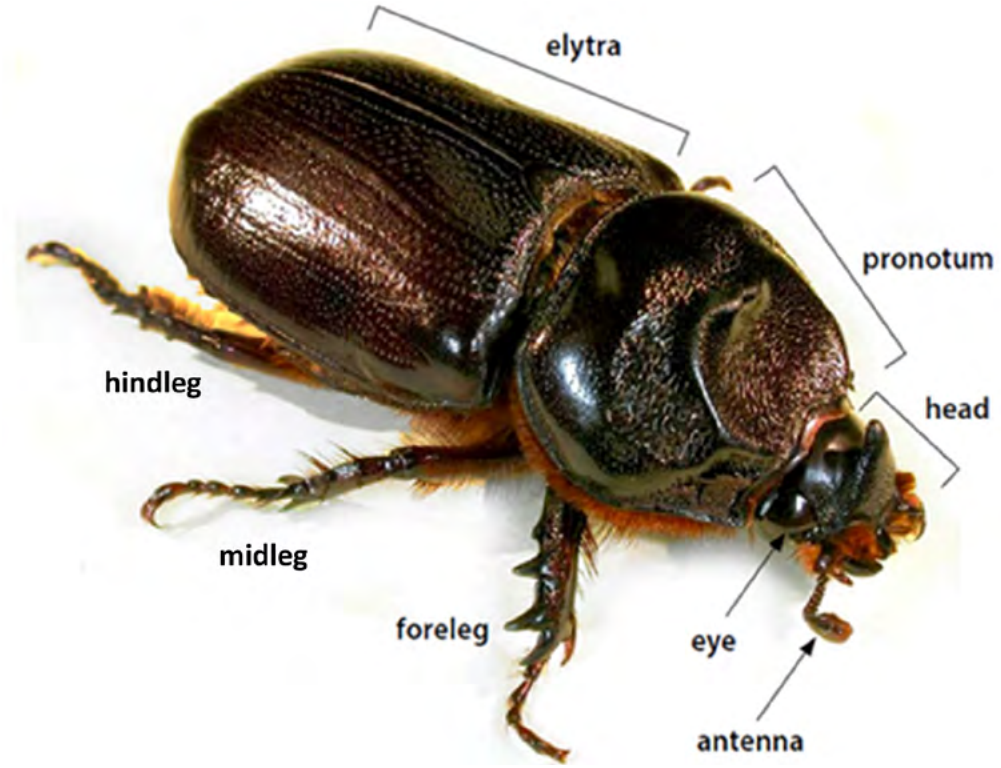


Figure 2.3 Adult CRB.

The head supports a broad horn (of variable size) extending from the clypeus (above the mouthparts). Males generally have a larger horn than females, but horn size cannot be used as a definitive differentiating characteristic. Club-like lamellate antennae, typical of the Scarabaeidae, extend laterally concealing the strong mandibles which are used for rasping. The relatively small maxillary and labial palps can also be observed in the lower (ventral) area surrounding the mouth (not visible in Figure 2.3). The eyes are set into the sides of the head in front of the broad pronotum which extends across the body. The pronotum covers the first thoracic segment from which the broad, toothed forelegs extend. The remainder of the upper (dorsal) surface is covered by the elytra. These are the sclerotised, protective forewings and must be lifted for the insect to take flight using the rear pair of wings. The bulk of the body comprises the segmented abdomen which contains the gut and reproductive structures. To determine the sex of the insect, a good indicator is to examine the posterior section of the abdomen (pygideum): in male beetles, the posterior sternites are smooth, while in female beetles, the sternites are covered with fine hairs (Figure 2.4).

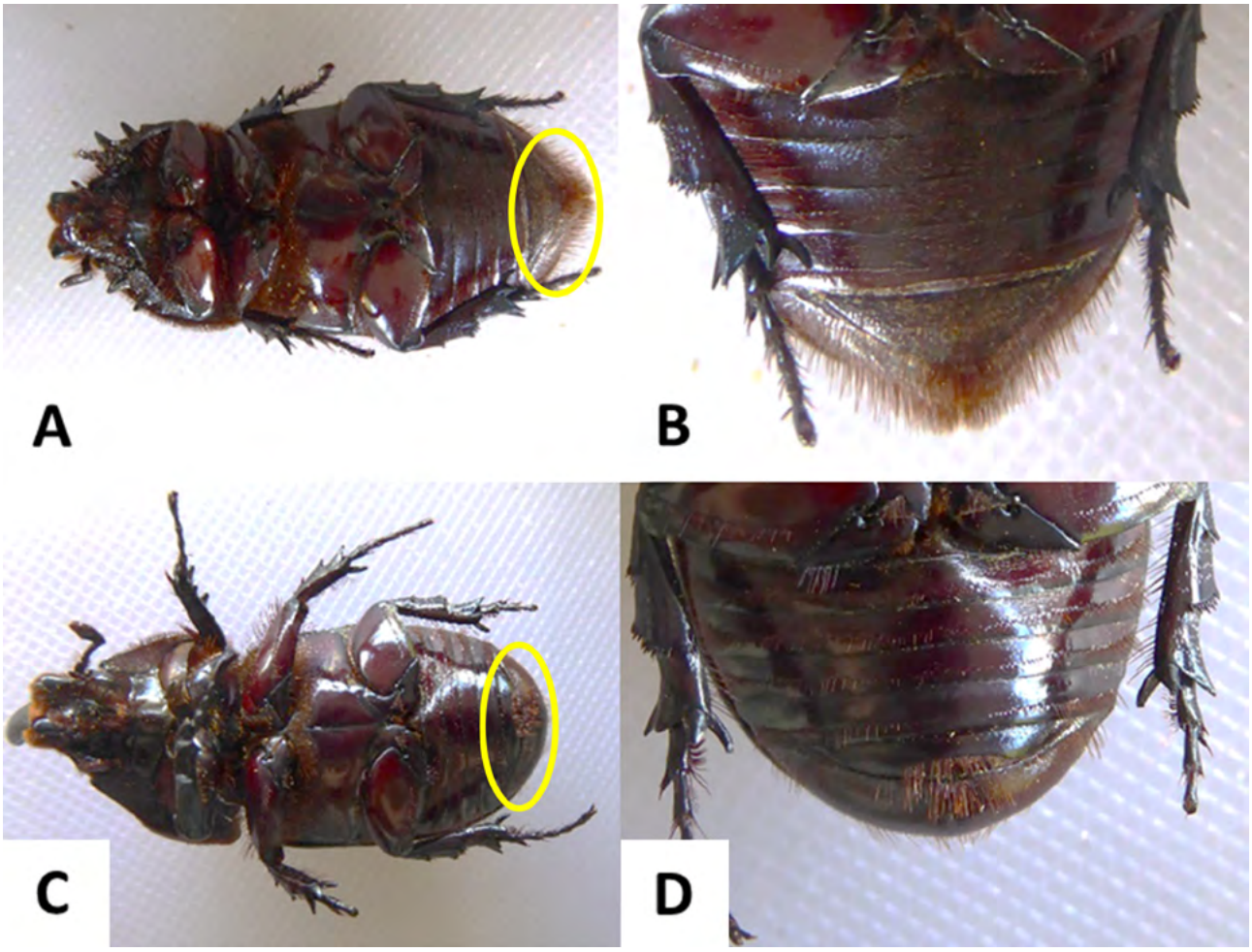


Figure 2.4 Differentiating male and female CRB. The pygidium (terminal section of the abdomen, yellow circle in A and C) of the female CRB is pointed and covered with fine hairs (A and B) while the posterior of the male is rounded and smooth with few hairs (C and D). Note that horn size does not distinguish between male and female CRB.

Adult beetles feed at the palm crown and are the damaging life stage (section 3.2). They are nocturnal, flying at night when they may be attracted to lights, but remain hidden inside palm crowns and breeding sites during the day.

Determination of whether a beetle is CRB or another species is largely dependent on the location of the find. In the central Pacific islands, there are no native or invasive dynastine species, which makes preliminary identification of the large black beetles and huge C-shaped larvae simple. Invasive flower beetles (Cetoniinae), chafer beetles (Melolonthinae) and ruteline scarabs (Rutelininae) may be present on some islands but can be readily differentiated on the basis of adult size, colour and other morphological characteristics. In the islands of the western Pacific, *O. rhinoceros* can be confused with other endemic dynastine scarabs (Bedford 1976). For example, *Oryctes centaurus*, *Scapanes australis*, *Trichogomphus* spp. and *Xylotrupes gideon* can occupy similar habitats. Adults can be identified based on external features, although males typically have more distinguishing features than females (Figure 2.5). Other life stages, particularly larvae, are often difficult to differentiate from CRB. Descriptions of cohabiting species and keys for identification are provided by Bedford (1974, 1976), Beaudoin-Ollivier et al. (2000) and Pallipparambil (2014). To confirm identity, specimens should be sent to the Plant Health Laboratory of SPC's Land Resources Division (https://lrd.spc.int/ accessed 9 June 2025).

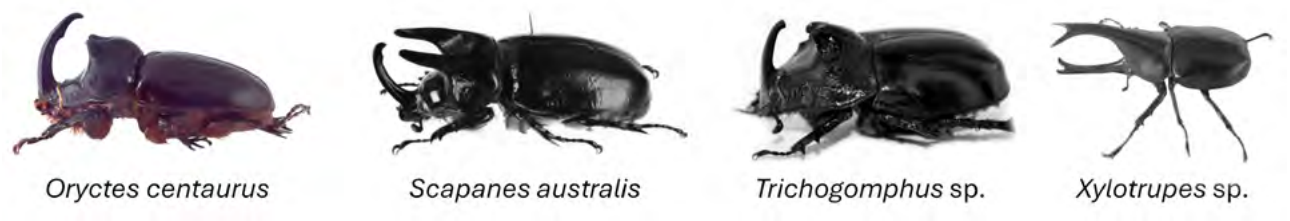


Figure 2.5 Adult males of several dynastine scarabs that may occur in similar habitats to CRB.

Photo credits: O. centaurus EL Engasser, https://idtools.org/scarab/index.cfm?packageID=2201&entityID=10469, other dynastine species Macfarlane 2018

If a new invasion of CRB is suspected, it is essential that the local biosecurity authority and/or Ministry of Agriculture is informed. This will ensure that the presence (or absence) of CRB can be confirmed, with appropriate action taken to contain and control any new incursions before the pest spreads further. When a new CRB invasion is confirmed by local authorities, it is recommended that the Pacific Plant Protection Organisation Secretariat at SPC be notified (https://www.ippc.int/en/ippc-community/regional-plant-protection-organizations/pacificplantprotectionorganisation/; accessed 9 June 2025).

2.2 Sample collection and handling

CRB adults or larvae are required for identification, ecological studies and assays with chemicals or biological agents for control. In all cases, careful handling and labelling of specimens is required for subsequent evaluation. Insects may be collected from field sites (damaged palms, heaps of compost) or from traps. The collector will need an insulated container (a "chilly bin" or picnic cooler), frozen blocks to keep it cool, and several containers or individual tubes for the insects (Figure 2.6; guidelines for live beetle or larvae collection are in Annex 1).



Figure 2.6 Collecting live CRB. After careful collection from the field, beetles should be placed in individual tubes in a cooler before transportation to the laboratory. A freezer pad or bottle of frozen water can be used to keep the interior cool.

To date, no morphological characteristics have been identified which can be used to differentiate haplotypes of CRB, therefore genetic methods must be used. Tissue samples must be collected from fresh CRB specimens and preserved appropriately for DNA analysis. Isolation of DNA and haplotype identification require specialised facilities. Please contact the SPC Plant Health Laboratory, mentioned above, regarding shipment of specimens for genetic analysis. Annex 2 gives detailed guidelines for collection, preparation and shipment of CRB samples.

In all cases, the collection should be given a specific recording number and date. The location of each collecting site should be recorded by GPS, and a photograph or written description made of the site. It is important to note whether the insects are taken from standing palms or from compost, the species of palm, the level of damage and density of palms. Adult beetles can also be collected from pheromone traps. For repeat samplings of a specific site, it is only necessary to record factors that have changed if data are maintained in a central database. It is important to retain both paper and electronic records for future reference. Electronic records should be backed up to the cloud or other secure data storage device, to protect against accidental data loss.

The precise manner of sample collection will depend on the purpose of the exercise. For a general collection where the objective is only to describe the population, insects can be combined in a container for later differentiation by species, sex, size, etc. It is important to place larvae into a container with sufficient organic matter substrate (sawdust, compost) to avoid damage and cannibalism. If the insects are being collected for further rearing to be used in assays or for studies of pathogens, it is important that they are placed in individual tubes (25 mL specimen jars or similar) until further examination. For all collections, it is important to keep the insects out of direct sunlight and to avoid overheating the tubes. Conversely, the insects should not be frozen or kept under refrigeration for long periods. Under natural conditions, CRB are long lived. Therefore, high death rates after collection are unusual and usually associated with the conditions of collection. Larvae in particular are sensitive to bruising and damage. If handled roughly, bruising will be noted a few hours after collection and the larvae will become sluggish, die and turn an intense blue/black colour within a few days. Death rates (the proportion of insects that are dead after seven days) among batches of collected larvae should be recorded as these will provide an indicator of collection success.

2.3 Damage recognition

CRB adults feed on plant tissues of the developing leaves and meristem of several tropical monocot plants, including palms, particularly coconut and oil, as well as banana, sago and pandanus. For a list of adult food plants, refer to Gressitt (1953).

The anatomy of palms is described by Broschat (2013). In both coconut palm and oil palm, the CRB adults fly to the palm and force their way down between the leaf axils until they find a position where they can burrow into the plant. Feeding and damage to coconut palm was described by Young (1975). CRB damage is characterised by straight cuts across the fronds of palms which give the appearance of V-shaped notches in the leaflets of the emerged fronds as if cut with scissors (Figure 2.7A). The damage to the palm crown is caused by adult beetles walking down the frond axil until they are lodged between the open frond and the growing spear (Figure 2.7B). Securely positioned, they will bore through the spear, cutting leaflets in the compacted unemerged frond before

they reach the soft tissue of the young underdeveloped fronds. In the most severe cases, beetles will feed on soft leaf tissues (Figure 2.7C) until they reach the meristem, leading to destruction of the fronds and even death of the palm. The effect of multiple beetles feeding on the developing fronds was illustrated by Gressitt (1953) and is shown in Figure 2.8. It is common to see mature palms with varying levels of damage side by side (Figure 2.9). Juvenile palms are quickly overcome when attacked repeatedly by CRB (Figure 2.10).

While severe CRB damage to mature coconut palms is very recognisable, there are other dynastine scarabs (primarily *S. australis* and *O. centaurus*) in the Pacific that cause damage, particularly to juvenile palms, that may be confused with CRB activity (Stapley 1973). If the identity of the insect causing palm damage is uncertain, collecting live insects for identification is essential (see section 2.2 and Annex 1).



Figure 2.7 (A) Coconut palm showing distinctive V-shaped notches in the fronds caused by CRB adult feeding. (B) CRB adults in the axil between the emerging frond and the spear. (C) CRB adults feeding in the soft core tissue of the palm.

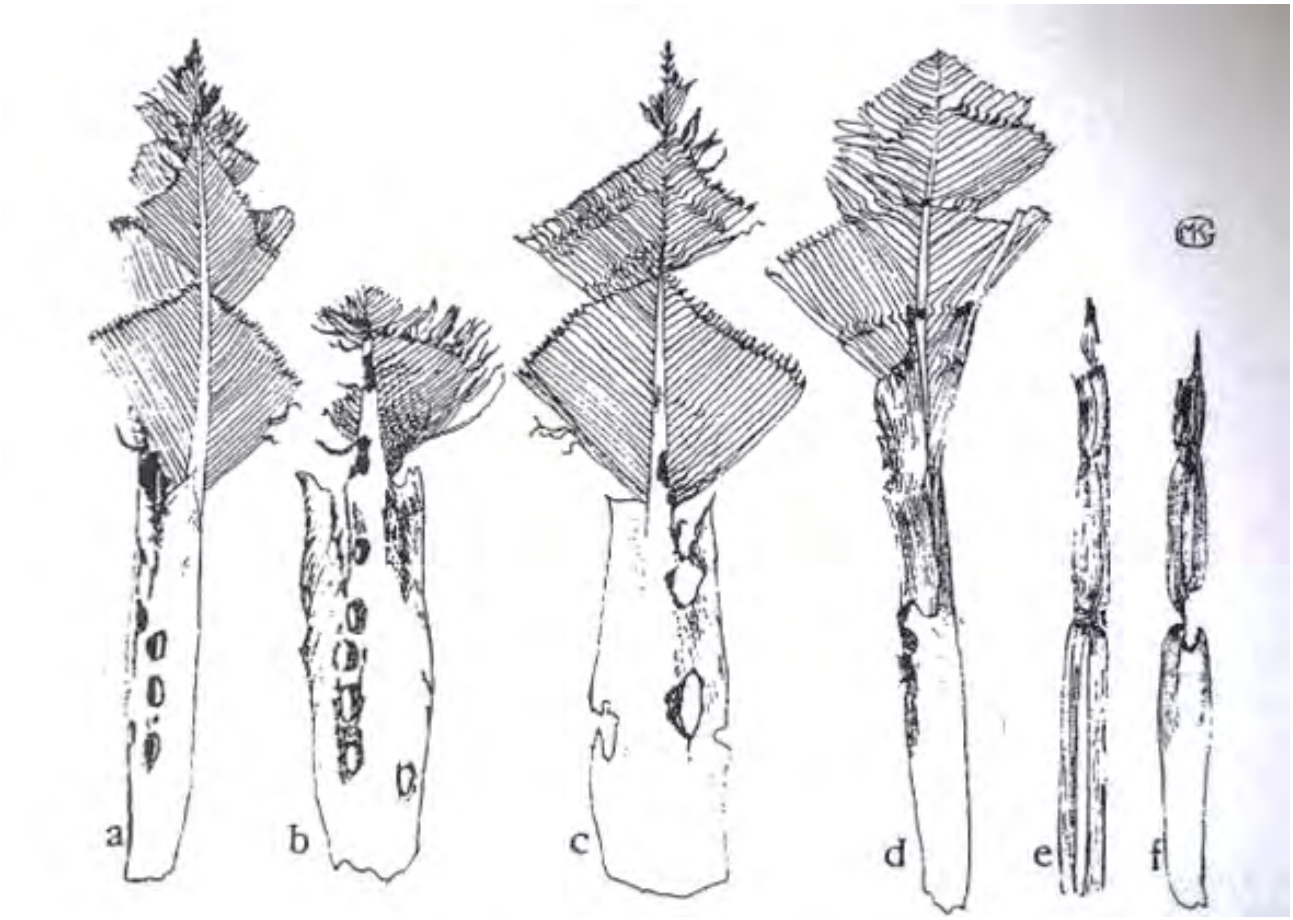


Figure 2.8 Damage from CRB to the central growing point of the palm (Gressitt 1953).



Figure 2.9 Coconut palms damaged by CRB near Honiara, Solomon Islands. Note the range in damage between adjacent palms.



Figure 2.10 (A) Juvenile oil palm with CRB damage. (B) Base of juvenile oil palm showing boreholes from CRB.

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Source: Iriarte et al 2015

3 Surveillance and monitoring of CRB populations

This section describes methods used for CRB surveillance (the detection of new invasions into a country or region) and CRB monitoring (measuring relative abundance and/or impact of established CRB populations). When a new invasion is detected, a delimitation survey is needed to determine the geographic extent of the invasion and to inform the emergency response (section 3.1). When dealing with established CRB populations, baseline surveys provide a benchmark to measure the success of control measures used to manage the pest (section 3.1). Both surveillance and monitoring rely on visual observation of palm damage (section 3.2) and the use of pheromone traps to capture adult CRB (section 3.3).

3.1 Delimitation and baseline surveys

In the event of a CRB outbreak, it is important to establish the border of the infested area quickly and to gather information in order to decide what action should follow. When the CRB outbreak is a new incursion into a country or region, the size and severity of the outbreak must be defined quickly, to determine if eradication should be considered or if biosecurity authorities should focus on regional containment and long-term mitigation through IPM strategies. When the outbreak is in a region where CRB has been well controlled previously, the survey may identify the cause of the outbreak so that management strategies can be improved.

The delimiting survey starts from the area where the incursion was reported. Actions to take are listed below.

1. Take pictures of the pest, symptoms, affected plants and areas. If the pest has not been identified formally, collect samples to confirm it is CRB (Annex 1).
2. Establish when the pest reached the area and potential mode of spread to the area. This involves visual assessment of CRB damage to palm crowns (section 3.2) to estimate how recently damage occurred as well as discussion with the local community to understand potential entry pathways for CRB and when local people first saw palm damage.
3. Assess the speed of the pest's dispersal based on the observed spread out from the most likely point of entry. This can be estimated based on the approximate time of arrival at the point of entry and the distance from the entry point to the infestation boundary.
4. Map boundaries and estimate the size of both the area already infested and possible areas into which the pest could spread.
5. Assess the area currently covered by the host plants within the sites concerned.
6. Assess the estimated financial loss and social damage that may be caused by the pest if it spreads to the whole endangered area.
7. Identify plants, plant products or other articles whose movement out of the infested area needs to be regulated in order to contain the pest.
8. Identify how these plants or other articles could spread further (wind, human transport by boat, aircraft, private and public vehicles).
9. Identify owner(s) of the plants, plant products or other articles, and explain the situation to them.
10. Assess the possibilities of stopping the pest from spreading further.
11. Assess the feasibility, cost and possible problems of containing, eradicating or managing the pest outbreak.
12. Identify how and where infested plants and/or products could be treated or disposed of.
13. Inform local authorities, extension officers and producers of host crops about the pest.
14. Recommend local staff who would need to be engaged in further actions.

A baseline survey is recommended before mass release of biological control agents or beginning a sanitation programme. The purpose of a baseline survey is to document the severity of CRB damage so that the impact of control measures can be evaluated effectively in the future. Baseline surveys should target the immediate area where control measures will be applied, using the damage assessment methods described in section 3.2. It is helpful to combine damage assessment with pheromone trapping to monitor the CRB population, if the resources are available to support deployment and maintenance of the traps (see section 3.3).

3.2 Damage assessment

Damage assessment is an essential part of delimitation and baseline surveys for CRB. It is also an important tool to monitor impacts from ongoing CRB management. The distinctive notches produced by feeding adults are a clear marker of CRB and will often be the first indicator of their presence. The assessment team should identify damaged palms, record their location with GPS, and record the level of damage with photographs. The site should be mapped for damaged palms, and the team should proceed beyond the outbreak area for 1–5 km until no further damage is noted. The results should be discussed with the local community, and a reporting system will need to be established to monitor potential spread. If not completed already, collect live beetles and/or larvae to confirm it is CRB and provide evidence of establishment (see section 2 and Annex 1 for collection methods).

Damage to mature coconut palms can be quantified using binary, 3-point, or 5-point damage scales (Figure 3.1). The 5-point scale (undamaged, low, medium, high damage, dead) reflects the impact of CRB damage on coconut production. The relationship between palm frond loss and yield reduction was originally quantified by Young (1975), and his data describes what is essentially a linear relationship between the two (Figure 3.2). When the category boundaries of the 5-point scale are superimposed on this relationship (Figure 3.2), it demonstrates that yield declines substantially once the medium damage level is reached, decreasing even further as damage increases. If the majority of palms are rated as medium damage or above, coconut production in the affected region will be much lower than before the CRB outbreak. This has led to the recommendation that the long-term target for successful CRB management and associated palm recovery is a median of low damage for the region. This also reflects observations of CRB damage where this pest is generally well controlled, such as Fiji.

The effectiveness of these damage scales was evaluated by Mansfield et al. (2024), whose recommendations for scale type and sample size are summarised in Table 3.1. For a delimiting survey, the binary scale is sufficient to delineate the area of infestation. Use of the binary scale is also recommended for survey teams that are less experienced with damage assessment. Baseline surveys prior to sanitation activities should use the 3-point scale so that the locations of dead palms can be mapped for removal. Baseline surveys



Binary scale

Undamaged	Damaged

3-point scale

Undamaged	Damaged	Dead

5-point scale

Undamaged	Low damage	Medium damage	High damage	Dead

Figure 3.1 Binary, 3-point and 5-point scales with exemplar photos for assessment of CRB damage on coconut palms (Mansfield et al. 2024). Categories for the 5-point scale are: Undamaged = no damage symptoms evident; Low damage = notching or tip damage, < 20% foliage loss; Medium damage = multiple fronds affected with notching and breakage, 20–50% foliage loss; High damage = multiple fronds affected with notching and breakage, > 50% foliage loss; Dead = palm dead or growing point destroyed.

to support biological control programmes and future evaluation of IPM should use the 5-point scale, subject to the availability of trained personnel.

As an example, palm damage was compared on Efate, Vanuatu, between September 2019, shortly after CRB invaded the island, and July 2022, by which time the invasion was well established. The increase in damage is very apparent, with the median level of damage increasing from low (2) to medium (3) using the 5-point scale and the proportion of undamaged palms decreasing from 40% to 4% (Table 3.2). The impact of CRB on Efate, including mapping of palm damage throughout the island, is described in more detail by Paudel et al. (2023a).

In addition to assessment of the entire palm crown, monitoring new growth for change can be a useful indicator of recent CRB activity. A healthy coconut palm growing in favourable environmental and nutritional conditions will produce fronds at approximately monthly intervals and hold approximately 20–25 fronds before they senesce and drop. Additionally, from formation in the growing point to emergence takes about 3 months. By making an assumption based on these figures, or modifying them for growth in local conditions, it allows us to use the appearance of the coconut palm crown to estimate the age (from formation) of fronds on the palm (Figure 3.3). Damage to the lower half of the crown is older than damage in the upper half of the crown.

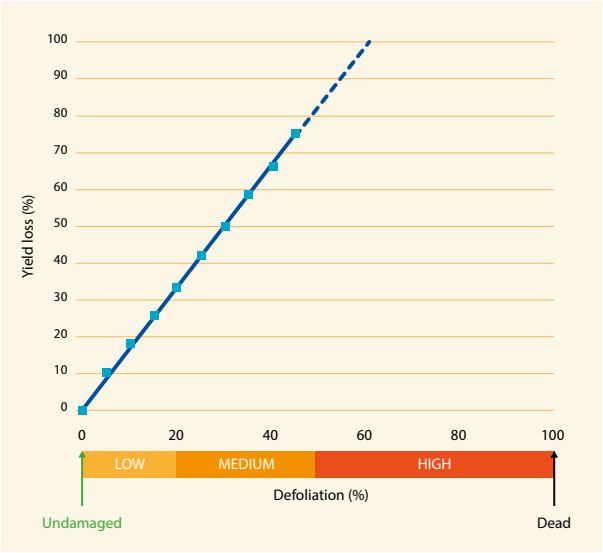


Figure 3.2 Relationship between loss of coconut palm fronds due to CRB damage and loss of coconut yield (derived from Young 1975). Defoliation–yield loss relationship overlaid with the 5-point damage scale. Extrapolation of the linear relationship (dashed line) suggests nut production will cease after approximately 60% defoliation.

Estimating the age of first attack based on crown position can give clues to the arrival of a CRB incursion. It also indicates success or failure of a CRB control action. Recording damage, or its absence, to the topmost four fronds gives an estimate of CRB feeding and damage that has occurred in the previous six months. This measure can be used to assess the effectiveness of recent control actions against the beetle in the short term, particularly by local communities, alongside more quantitative damage assessment provided by the 5-point scale.

Mature oil palms also suffer attack from CRB that can impact on yield, although immature palms are more vulnerable to damage (discussed below). Where CRB is present, monitoring of CRB damage is recommended as part of the general oil palm IPM strategy. For CRB populations in PNG and Solomon Islands, control measures are recommended if the amount of new damage to oil palms has increased above 5% compared with the previous assessment.

Juvenile coconut and oil palms, particularly those that are less than 5 years old, are more susceptible to CRB damage than mature palms. These young palms can die rapidly from repeated CRB attacks, so close monitoring for CRB damage is strongly recommended. Control measures should be applied quickly once damage is detected on young palms.

Table 3.1 Recommendations for CRB damage assessment based on the purpose of the assessment (Mansfield et al. 2024).

	Recommendations		
Purpose and scenario description	Frequency	Scale	Minimum sample size
Detection of new CRB outbreak Border surveillance to detect new CRB incursion into country or region where CRB is not present	Every 3 months	Binary	At least 50 palms around each potential point of entry
Delimitation survey after new CRB outbreak Define geographic extent of new CRB incursion to support response efforts	Conduct survey as soon as possible after initial detection	Binary	Survey all palms in target region until incursion boundary identified
Support sanitation programmes for CRB management Determine locations of dead palms to be removed to reduce CRB population	Conduct survey in target region(s) before starting sanitation activities	3-point	Survey all palms in target region
Detection of worsening CRB damage Detection of potential new CRB variant in country or region where CRB is well established OR detection of an increase in local CRB population	As needed for regional biosecurity; every 6 months for plantation management	5-point	At least 200 palms in target area, e.g. coconut plantation
Detection of palm recovery from CRB damage Determine efficacy of response measures against CRB	Baseline survey prior to CRB response activities then repeat every 6 months	5-point	At least 200 palms within each area subject to CRB management

Table 3.2 An example comparison of palm damage by CRB using the 5-point scale on Efate, Vanuatu. In September 2019 CRB was first reported from Efate and the infestation was limited to a small part of the island. In July 2022 the severity of palm damage and the CRB-infested area on Efate had increased significantly compared with the initial assessment.

	Assessment date **September 2019**	**July 2022**
Damage score	**Number of palms (%)**	**Number of palms (%)**
1 Undamaged	51 (40.5)	12 (4.0)
2 Low damage	53 (42.1)	72 (24.2)
3 Medium damage	17 (13.5)	80 (26.9)
4 High damage	3 (2.4)	56 (18.9)
5 Dead	2 (1.6)	77 (25.9)
Median damage score	2	3
Total palms surveyed	126	297

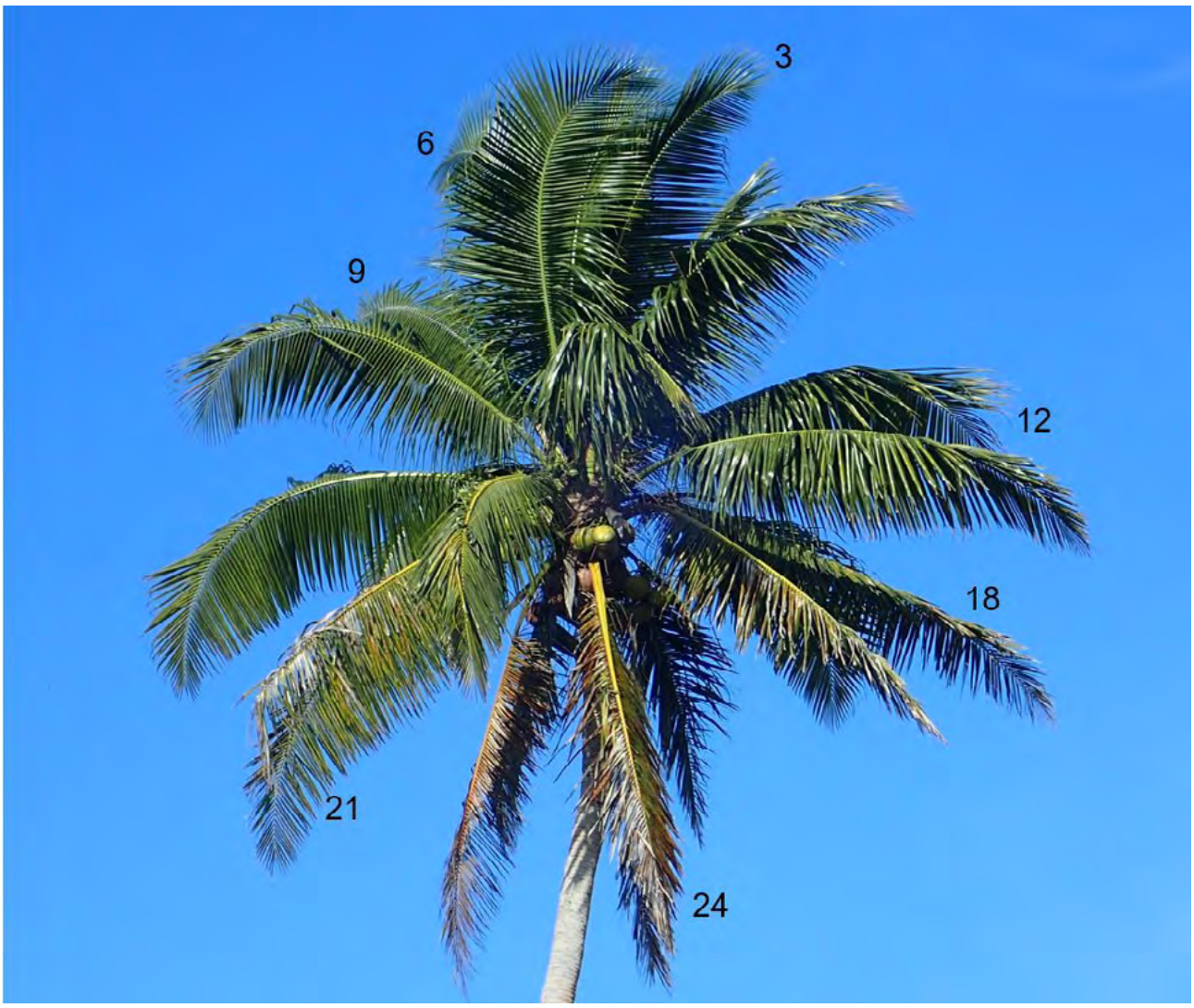


Figure 3.3 Estimated age in months (numbers) of fronds on a mature coconut palm. Newly emerged fronds unfurl from the growing point at the centre-top of the palm crown, gradually shifting downwards over time as new fronds unfurl. The oldest fronds hang down close to the trunk. CRB damages fronds before they unfurl from the growing point, so damage to new fronds is much more recent than damage to old fronds.

3.3 Pheromone traps

CRB trapping can be used for different purposes. These include surveillance for early detection, monitoring a population over time, or to assist with control of a CRB population. In all cases the trap needs to be attractive for the beetle to enter and strong enough to contain the insect once it is inside. Traps for CRB are usually used in combination with pheromone lures that release the CRB aggregation pheromone ethyl 4-methyloctanoate (E4-MO). Adult males produce this pheromone naturally to attract both females and males to feeding sites inside the palm crown. E4-MO has been used for more than 30 years and is produced commercially as a sachet, which can be placed in a trap (Hallett et al. 1995; see Table 3.3 for a list of suppliers).

Table 3.3 Commercial CRB pheromone products currently available (Paudel et al. 2023b).

Manufacturer	Product	Description
AlphaScents, USA alphascents.com	ORYRHI	E4-MO in a coaster lure packet
ChemTica, Costa Rica chemtica.com	P046-Lure	E4-MO in a blister lure packet
ISCA, USA iscatech.com	Splat RB	E4-MO in a sticky matrix
ISCA, USA iscatech.com	Hook RB	An attract and kill product containing E4-MO and cypermethrin in a sticky matrix
Laksitha Agro Biotech, India	Sun Agro	E4-MO in a coaster lure packet
Harmony Ecotech Ltd., India harmonyecotech.com		E4-MO in a coaster lure packet
Green Revolution, India	Beetle lure	E4-MO in a coaster lure packet
Gaiagen Technologies (Formerly, Pest Control India), India gaiagentechnologies.com	RB-lure	E4-MO in a coaster lure packet
Indian Agri Farm, India indianagrifarm.co.in	Rhinoceros beetle trap lure	E4-MO in a coaster lure packet
Russell IPM, UK russellipm.com	*Oryctes rhinoceros*	E4-MO in a blister lure packet
NovAgrica, Greece Novagrica.com	Rhinogel	E4-MO in a blister lure packet

The use of pheromone traps for CRB management was reviewed by Paudel et al. (2023b). This review recommended that pheromone traps, particularly bucket and pipe traps, were most useful for surveillance to detect new incursions of CRB, and monitoring of new invasive populations. Mass trapping is widely used in commercial plantations in the native range of CRB but the benefits of mass trapping in the invasive range remain unclear.

This section focuses on pheromone trap construction, deployment, maintenance, and how to standardise trap catch data over time.

Traps for surveillance and monitoring need to be robust, inexpensive, attractive to beetles, difficult to exit and simple to service. Bucket traps, often with vanes, have been used in Guam and Hawai'i, where thousands of traps have been distributed and used for delimitation of the spread of a CRB invasion and to monitor success of control activities. Bucket traps have also been used extensively for monitoring throughout the Pacific Islands and Southeast Asia (Figure 3.4). These traps consist of a bucket with a lid that has holes to permit beetle entry, and smaller drainage holes at the bottom to let rainwater out. The pheromone sachet is hung inside from the lid of the bucket. Bucket traps are usually hung from a convenient tree branch so they can be moved easily to new locations.

A more costly alternative to the bucket trap is the pipe trap (Figure 3.4), which was developed by the oil palm industry in PNG and has been widely used (Morin et al. 2001). It is made from a 2 m section of PVC piping secured to an iron bar above a collection tray or bucket. Two "windows" are cut into the pipe near the top, which allow entry of the beetles before they fall into the collection chamber at the base of the pipe. A pheromone sachet is hung within the pipe near the entry windows. Pipe traps are fixed in surveillance sites and suitable for long-term monitoring.





Figure 3.4 Bucket trap (top) and pipe trap (bottom) for capturing adult CRB.

Both bucket and pipe traps are prone to theft or vandalism once deployed in the field. It is recommended that these traps be placed out of sight from the road and preferably at locations where local landowners, businesses or community organisations have agreed to look after them. Schools and churches are often willing to host traps. Staff involved in the CRB response may be able to have a trap at their home.

Traps should be sited in a position attractive for the flying beetles (on a ridge or in a specific tree) with the aim of obtaining a consistent representative sample of beetles. The trap position should be fixed (by map or GPS) and recorded. The traps should be cleared regularly, with beetles differentiated into males and females and numbers recorded. Traps used for surveillance to detect new incursions, or if beetles are needed alive for diagnostic purposes, such as DNA analysis, should be emptied at least once a week and the beetles handled carefully when transported back to the lab (see Annex 1). If the trap is used for monitoring beetle numbers in established populations, the bucket should be emptied every 3–4 weeks, and the collected beetles destroyed after counting and recording the catch. Pheromone sachets should be replaced when they have dried out. Pheromone decay depends on local weather conditions; sachets may last up to 4 months but checking the sachet at least every 2 months with replacement as needed is recommended to maintain attractiveness to the CRB.

The number of traps deployed should be considered carefully in relation to the target area and the resources available to maintain the traps regularly and to collect catch data in a timely manner. In commercial plantations, one trap per hectare is the highest density recommended for CRB management, but a lower density (one trap every 2–3 hectares) will still provide useful information. For surveillance at border entry points, such as air and seaports, frequent checks are essential to maximise

the likelihood of detection. The number and placement of traps should take this into account. If a new CRB incursion is detected at the border, biosecurity authorities need to be informed quickly so that an emergency response can be initiated.

The dates when traps were cleared, when pheromone sachets were replaced, and beetle counts need to be recorded consistently. In the office, trap catches should be analysed, mapped if possible, and summarised. Trap catch should be converted to beetles trapped per day (= 24 hours), as this figure can be compared between sites and dates for the same type of trap. While traps provide only a relative measure of CRB density, regular monitoring will indicate long-term trends (either up or down) in the surrounding beetle population. This information, combined with palm damage assessment, will assist with decisions regarding control measures.

3.4 References

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4 Control measures for CRB

CRB is native to Southeast Asia, where the distinctive V-shaped notches caused by adult feeding are seen frequently in coconut palms, but the damage is seldom sufficient to warrant control due to the presence of co-evolved natural enemies. In its native range the insect becomes a pest, causing severe damage, where there is an abundance of old palm trunks and organic matter, such as that left after cyclones or after felling of senile palms for establishment of new plantations (Figure 4.1). In these conditions, female beetles can fly into the area and lay eggs in the organic matter, with high numbers of larvae developing in the decaying material. When this generation emerges, the numbers of beetles and the continuing abundance of food resources can lead to a population explosion, with emergence of very high numbers in the second generation. This can cause a great deal of damage to nearby palms, even killing many, which leads to further substrate availability and an ongoing problem from the beetles.

Some of the measures that are widely used to manage CRB populations are:

- sanitation
- biological control
- chemical control
- mechanical control
- internal biosecurity
- eradication
- integrated pest management (IPM).

4.1 Sanitation

Sanitation involves removing organic matter sources, and is especially important to prevent establishment of new populations and to limit damage from established populations. CRB larvae feed and grow in rotting organic matter. The life cycle can be disrupted and populations reduced if bulk sources of organic matter are removed or at least reduced.

Sanitation methods are reviewed in Godshen (2015) and the sanitation programme carried out in Guam has been described by Smith and Moore (2008). Sanitation for CRB control is labour intensive and requires community support for success. It is, however, a highly effective tool that complements other control measures. Where sanitation is part of the response to a recent invasion by CRB, it is important to keep thorough records of clean-up activities to ensure systematic coverage of the target region. In any programme to control CRB, a sanitation plan should be developed and carried out, as outlined below.

The goals for a sanitation programme are: (i) to remove dead standing palms so that these represent less than 5% of palms in the target region; (ii) to ensure sanitation practices are incorporated during plantation renewal for coconut and oil palms; and (iii) to encourage local communities to manage organic waste actively to minimise CRB breeding.

Disposal of dead standing palms, felled palms and palm stumps

CRB adults are attracted to dead standing palm trees that have begun to rot from the crown. Females will lay their eggs in the rotting palm trunk and the developing larvae will feed on the decaying fibres near the top of the trunk, which starts to decompose in the centre forming a protective tube for larval development. As the larvae increase in size and mandible strength, they penetrate further down the trunk leaving a column of frass and cut fibres suitable for development of more early instar larvae.

In some situations, larvae may develop in the crown of live palms. This only occurs where there are large accumulations of organic matter in the frond bases (Moore et al. 2014). This organic matter should be removed manually from the frond bases where possible, if there is evidence of CRB breeding activity.



Figure 4.1 (A) CRB larvae feeding on the outer layer (cortex) of a felled palm trunk in the early stages of decomposition. (B) High numbers of CRB larvae removed from a decomposing palm trunk.

Mature palm trees can fall after being weakened by fungal diseases (such as *Ganoderma* spp.), or be brought down by strong winds during cyclones. Dead standing palms and senile palms should be felled prior to replanting. The fallen or dead palms on the ground should be cut into pieces (using machetes, chain saws or machinery) and the pieces spread out to speed up decomposition and remove potential breeding sites. Burning palm waste is another option if local regulations allow this. The CRB response in Hawai'i provides a well-documented example of sanitation to control CRB (Coconut Rhinoceros Beetle Response – Hawai'i, nd). For oil palm plantation renovation, cutting the trunk into small lengths to accelerate breakdown is recommended.

The felling of palms will leave a stump which is suitable for development of larvae as it rots. In management of palm plantations in Asia where a zero-burning policy is in operation, ground cover is planted shortly after felling to cover the debris (stumps and chipped trunks) and make it less attractive to the flying beetles. Management of ground cover in Asia is reviewed by Sahid and Weng (2000). Legumes (*Calapogonium* spp., *Mucuna* spp. and *Pueraria javanica*) are commonly used as ground cover plants, as these will add nitrogen to the soil as well as covering the decaying trunks. Cover crops are used in a similar fashion for CRB management in oil palm plantations in PNG and Solomon Islands, particularly those plantations with certification from the Roundtable on Sustainable Palm Oil (RSPO). When piles of chipped palm waste are created during replanting, these are sown with the legume cover crop. There is potential to apply the fungus *Metarhizium majus* as a biological control agent during replanting (see section 4.2 below).

Similar principles for sanitation should be followed by villagers to manage damaged palms. That is, dead standing palms should be felled and trunks cut into smaller pieces to speed up decomposition. If available, *M. majus* can be applied to palm waste piles (see section 4.2).

Management of organic matter and compost

Heaps of organic matter, particularly palm debris, provide excellent food material for development of CRB larvae. Any deep piles of organic material will be attractive to the egg-laying females. Heaps of fronds or empty oil palm fruit bunches are particularly attractive. Sawdust from sawmills that process palm timber is also a favourable resource for beetle development. General compost, farmyard manure and even organic garbage, including household waste, can also provide sites for development of the larvae.

The first step in reducing the threat of beetles emerging from organic matter is management of the organic matter. Palm debris should be spread among the palms to break down rapidly and release nutrients rather than being piled in heaps. Compost or farmyard manure should be turned regularly, and larvae removed, or pigs and chickens can assist by eating exposed larvae. In urban environments, organic material is often gathered during environmental clean-up and composted, but this may provide a centre for re-infestation of the locality. Compost can be sterilised or fumigated to kill larvae; however, this process is energy-demanding and expensive. Sterile compost will also be susceptible to reinvasion. Where feasible, compost heaps can be covered with netting to trap emerging beetles as well as those coming in to lay eggs (Figure 4.2).

Tekken Netting

A gill net, called "*tekken*" in Chamorro, with a one-inch mesh measured knot to knot made from 0.25 mm nylon monofilament should be laid over piles of green waste such as palm/tree cuttings or decaying organic matter (Fig. 2). Green waste piles are very attractive to rhino beetles looking for a mate and/or egg-laying sites. A beetle trying to get in or out of the pile will become trapped when the monofilament drops into the gap behind its prothorax (Fig. 3), the same way fish are caught in gill nets.



Figure 2: Tekken net covering a large pile in fresh organic material.



Figure 3: CRB caught in Tekken netting

Figure 4.2 Use of Tekken netting to protect compost heaps from infestation by CRB (Iriarte et al. 2015).

4.2 Biological control

Biological control is the use of natural enemies (predators, pathogens or parasites) to suppress pest populations (Van Driesche and Bellows 1996). In its native range, CRB is attacked by a community of co-evolved natural enemies (reviewed by Bedford 1980 and Paudel et al. 2021), including pathogenic viruses and fungi, predatory carabid and elaterid beetles, and parasitic *Scolia* wasps. The relative impact of each natural enemy species on CRB within its native range is poorly known, but together they are generally effective at controlling CRB. Additional control strategies may be needed to complement biological control, particularly in coconut and oil palm plantations, when there is a build-up of natural breeding sites for CRB (see section 4.7). Incidental predation by pigs and chickens on CRB larvae can be useful for control of CRB larvae in household or community waste piles. Local species of generalist arthropod predators (centipedes, beetles and ants) may feed on CRB larvae; however, there is little evidence that this contributes significantly to CRB control (Hinckley 1967).

When CRB Clades II and III invaded the Pacific during the 20th century, CRB was the focus for a substantial biological control programme. The aim was to find one or more natural enemies in the native range that could be introduced to the invaded range to suppress CRB populations (Hoyt 1963). This process is known as classical biological control (Van Driesche and Bellows 1996). Of the many natural enemies introduced to the Pacific, very few predators or parasites established (Caltagirone 1981).

Only one pathogen provided significant control of CRB in the Pacific: Oryctes rhinoceros nudivirus (OrNV), discovered in Malaysia by Alois Huger. The history of the virus discovery and its use against CRB is summarised by Huger (2005). This virus infects mainly CRB adults, usually causing death after 3–8 weeks (Zelazny 1973). Infected adults are weakened prior to death so that they stop feeding, and their mobility and breeding are reduced. Larvae can also be infected with OrNV although less frequently than adults. Larvae die faster after infection, usually within 1–3 weeks, with third instars surviving longest (Zelazny 1972). Once established in Pacific countries invaded

by CRB during the 20th century, OrNV had a significant impact on CRB populations and reduced palm damage (Huger 2005).

Another approach to biological control of CRB is the use of biopesticides based on fungi from the genus *Metarhizium*, several species of which have demonstrated pathogenicity against both adult and larval stages of CRB. Different *Metarhizium* spp. isolates have been developed into commercial formulated biopesticides (Paudel et al. 2021), or are used as unformulated fungus-colonised substrate (Villamizar et al. 2024) that can be applied to CRB breeding sites in both the native and invaded range (Bedford 2013; Villamizar et al. 2024).

Collectively, OrNV and *Metarhizium* spp. are the most significant biological control agents for invasive CRB populations (Paudel et al. 2021). Recommendations for their use are described in the following sections.

Biological control with OrNV

The recent wave of CRB invasions has changed management because these new populations are not susceptible to the isolate of OrNV introduced originally to control CRB Clades II and III in the Pacific (Marshall et al. 2017). This has led to a new biological control effort to identify OrNV isolates from CRB's native range that are effective against these new CRB populations.



Figure 4.3 Adult CRB being dosed with an OrNV isolate to determine virulence.

The search for effective OrNV isolates has become complex due to interbreeding between CRB haplotypes where they co-occur in the invasive range and genetic variation between invasive populations (AgResearch unpublished data). The most effective approach is to conduct screening bioassays under laboratory conditions to determine which isolate(s) is most effective for the target CRB population (Figure 4.3). This requires investment in capability building and support for diagnostic testing to measure infection rates during the screening process. Access to pure OrNV isolates is also important, although raw gut macerates from infected beetles may be used in some circumstances. In-depth description of the isolate screening process is beyond the scope of this document, which focuses on practical implementation of IPM. To implement biological control using OrNV requires a mass release programme to establish the chosen OrNV isolate within the invasive CRB population, as described below.

OrNV cannot be applied in the field by itself – rather the virus must be carried inside an infected beetle, which in turn will infect other beetles (Figure 4.4). The aim of a mass release programme is to continue releasing infected beetles until the virus is widespread within the invasive population throughout the affected island, region or country. This means staff will need training in the collection, maintenance and dosing of adult CRB for release, and in the mass production of OrNV within CRB larvae and/or CRB adults (Annex 3).

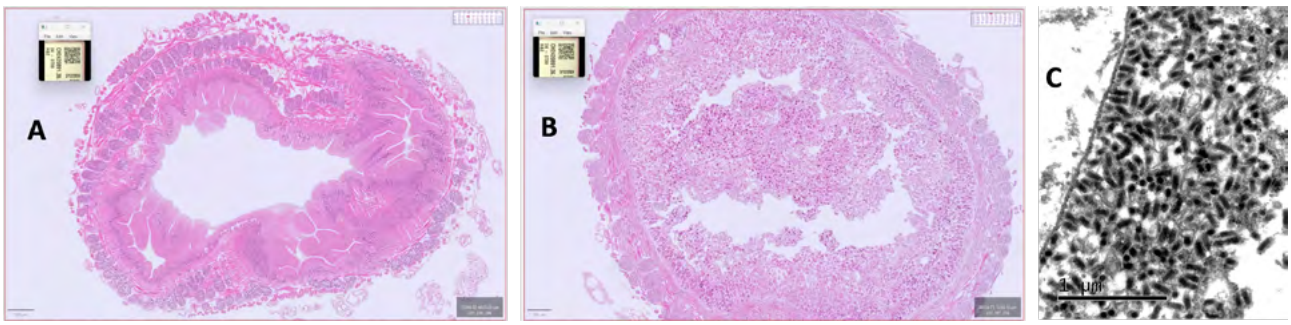


Figure 4.4 Cross-sections of CRB gut tissue that have been preserved and stained for diagnostic purposes. (A) Healthy gut tissue showing the central lumen empty of debris. (B) Virus-infected gut tissue showing the central lumen filled with damaged cells. (C) Electron micrograph of infected gut tissue showing fully formed virus particles (dark oblongs) filling the space between cells (photo: G. Barrera).

The selection of release locations is important because multiple releases are needed at each site and because there needs to be an established population of CRB at the site to allow OrNV transmission. Based on the historic OrNV mass release programmes in the Pacific, large numbers of infected beetles (hundreds) will need to be released for successful establishment and spread of OrNV (Marschall 1970; Bedford 1976). Once established, however, the long-term control provided by OrNV is worth the effort (Huger 2005).

Post-release monitoring at each release site is essential to determine if the virus has established sufficiently, allowing the release programme to move on to new sites, or if further releases are needed to increase the rate of OrNV infection. The recommended target is more than 50% OrNV infection on average across the invasive population, with OrNV present at more than 50% of sites sampled. Local staff will need specialist training in the collection and preservation of gut tissue samples from adult CRB to confirm OrNV establishment. If this is not possible, monitoring palms for signs of recovery is an indication of establishment that is also important to demonstrate success to local communities.

Expectations need to be managed during the release programme because OrNV establishment takes time (12–18 months with multiple releases at the chosen sites, and several years for widespread infection across a region). Palm recovery takes longer still, with localised patches of recovery expected a year after OrNV has established in an area, and full recovery taking several years. Where possible, deploying other control strategies alongside OrNV releases, i.e. an IPM approach (section 4.7), should speed up palm recovery compared with OrNV releases alone.

Biological control with *Metarhizium* fungus

Several species of *Metarhizium*, including *M. majus* (J.R. Johnston) J.F. Bischoff, Rehner & Humber (formerly *M. anisopliae* var. *majus*; Bischoff et al. 2009), *M. guizhouense* (Q.T. Chen & H.L. Guo), *M. lepidiotae* (Driver & Milner; Bischoff et al. 2009), *M. anisopliae* (Metschn., Sorokin), *M. pingshaense* (Q.T. Chen & H.L. Guo) and *M. brunneum* (Petch), have been evaluated against CRB under both laboratory and field conditions (Paudel et al. 2021; Prastowo et al. 2022; Villamizar et al. 2024). Among these, *M. majus* has demonstrated the highest virulence and efficacy (Velavan et al. 2017; Villamizar et al. 2024).

Some examples of commercial biopesticides based on *Metarhizium* and recommended for CRB control are presented in Table 4.1. Notably, the only product that specifies *M. majus* as the active ingredient is ORY-X, produced in Malaysia. Other products list *M. anisopliae* as the active ingredient; however, in some cases, the fungus may correspond to *M. majus* (which was formerly classified as *M. anisopliae* var. *majus*), as the label may not have been updated to reflect recent taxonomic revisions within the *Metarhizium* genus.

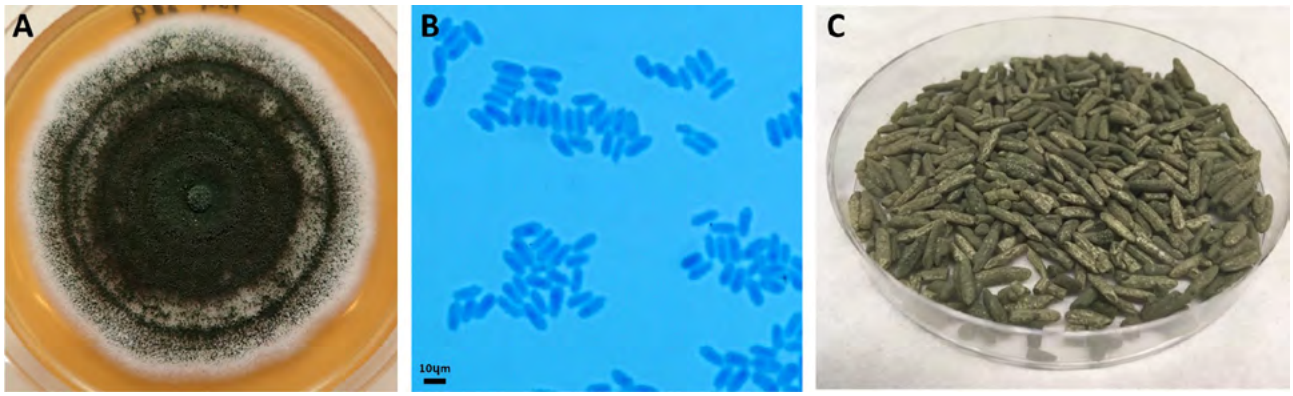


Figure 4.5 *Metarhizium majus.* (A) Colony grown on agar. (B) Fungal spores (conidia) observed under the microscope at 400× magnification. (C) Fungus-colonised rice.

The entomopathogenic fungus *M. majus* can be distinguished from other *Metarhizium* species by its large oblong-elliptical to banana-shaped spores with rounded ends (>10 µm in length, Figure 4.5). Its potential for CRB control was recognised very early (Friederichs 1913), when CRB first invaded Samoa in the early 1900s. It infects both CRB adults and larvae, however the humid microclimate of CRB breeding sites is most favourable to the fungus, so larval infection is usually more prevalent. Recently, Villamizar et al. (2024) identified and characterised a novel isolate of *M. majus* (F717) that is more virulent against CRB larvae and has shown potential during field experiments in PNG and Solomon Islands. This isolate is now being produced in bulk for field application in Solomon Islands. Another *Metarhizium* species, *M. pingshaense*, has been isolated from field samples in PNG but appears to only infect adult CRB not larvae (Villamizar et al. 2024).

The transient nature of most CRB breeding sites, which eventually decay to the point that the organic matter is incorporated into the soil and no longer attractive to female CRB, means that *M. majus* is best used as an augmentative biological control agent. The spores can be applied to breeding sites that cannot be removed, e.g. piles of empty fruit bunches at oil palm processing sites or used as mulch, and waste sawdust generated at sawmills, or to piles of chipped material that will be slow to decay fully, e.g. during plantation renewal or after felling dead palms. Depending on the rate of decay, repeated applications should be made every 4–6 months, until the organic material is fully decomposed (Villamizar et al. 2024). During renewal of oil palm plantations, application of *M. majus* is recommended on the waste piles of chipped palms just prior to sowing the legume cover crops (as described above in section 4.1). Knapsack sprayers or tractor-mounted mist blowers should be used to ensure good coverage with *M. majus* spores (water suspension with minimum concentration of 10^5 spores/mL and applied at 1 L/m^2 or 1000 L/pile; Annex 4).

Table 4.1 Commercially available biopesticides for CRB management in Asia.

Product name	Active ingredient	Formulation	Manufacturer	Country
ORY-X	*Metarhizium anisopliae* var. *majus*	Wettable powder	FGV Agriservices SDN VHD	Malaysia
Multiplex Metarhizium	*Metarhizium anisopliae*	Suspension Wettable powder	Multiplex Fertilizers Private Ltd.	India
Metarhizium anisopliae	*Metarhizium anisopliae*	Wettable powder	Agrikencana Perkasa	Indonesia
Super Meta (Mosa Meta)	*Metarhizium anisopliae*	Wettable powder	Mosa Mandiri Corporation	Indonesia
Metaribb	*Metarhizium anisopliae*	Wettable powder Granules	Riset Perkebunan Nusantara (Pusat Penelitian Bioteknologi dan Bioindustri)	Indonesia
Metatech	*Metarhizium anisopliae*	Wettable powder Sporulated rice	SVGroup	Thailand
Metarhizium anisopliae	*Metarhizium anisopliae*	Wettable powder	OrganicDews	India

An alternative approach that has been used during emergency responses to new CRB invasions is to create artificial breeding sites (ABS) that are then treated with *M. majus* (Annex 5). These ABS are open boxes filled with organic material suitable for oviposition by CRB. The aim of this strategy is to attract female CRB to lay eggs in the ABS, where the larvae will become infected and adults leaving the ABS may carry fungal spores with them to infect CRB elsewhere. For this strategy to be effective, it is crucial that the ABS are maintained regularly. The fungus should be reapplied every 4–6 months to ensure inoculum is maintained in the breeding material (Villamizar et al. 2024), and the quality of the breeding material needs to be monitored to ensure it is still attractive to CRB (Annex 5). This may require addition of new breeding material and/or adding water to maintain suitable moisture levels. The breeding material needs to be slightly damp to the touch, not dry or waterlogged. Inadequate maintenance will mean ABS become sources of healthy beetles, rather than sources of fungal infection, which is not helpful for CRB management.

Whether *M. majus* is applied to natural or artificial breeding sites, it is important that the product applied is of high quality, with an effective concentration of viable fungal spores (at least 1×10^8 spores per gram of substrate) and is not contaminated with unwanted micro-organisms. This is particularly important if local mass production facilities are to be established as part of CRB management. Staff will need training to ensure the quality of the final product. It is essential that production facilities are separated from other pest management work to avoid cross-contamination with fungal spores.

Application in the field needs to ensure adequate penetration of the breeding site material so that the spores are not exposed to the harmful effects of heat and sunlight. In ABS, mixing of the breeding material may help ensure spores are dispersed throughout the ABS, not concentrated in small patches. Infection rates can be monitored by checking the ABS or subsamples from treated breeding sites for infected larvae and adults (Figure 4.6). Tracking fungal infection in the environment beyond the immediate application site is time-consuming and may not be feasible unless significant resources are available for this purpose.

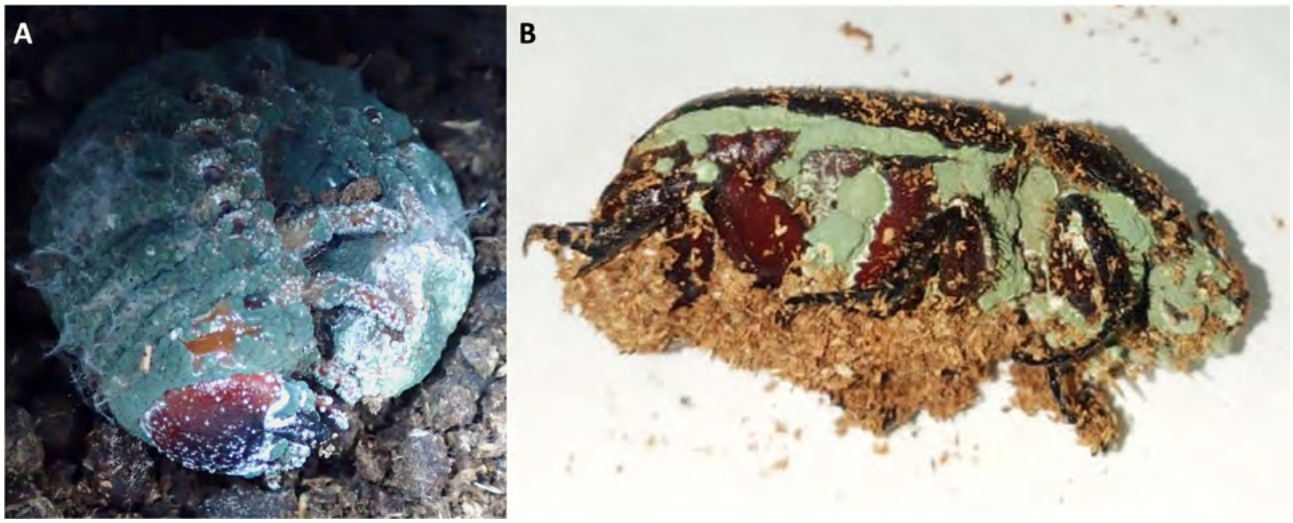


Figure 4.6 (A) CRB larva and (B) adult showing green spores indicative of *Metarhizium majus* infection (Villamizar et al. 2024).

4.3 Chemical control

Chemical control has limited application for CRB management, although it can be useful for protecting immature palms. Synthetic pyrethroid insecticides are used to prevent the adult beetle from damaging the spear and growing point on young palms. For example, cypermethrin is recommended to protect young oil palm replants in CRB-infested areas, but only until the palm starts fruiting as the insecticide can damage the beneficial pollinating weevil, *Elaeidobius kamerunicus* (Ismael et al. 2009). Moore (2013) also tested cypermethrin for control of CRB in Guam and found it effective when applied to young coconut palms.

For mature oil palms, trunk injection with thiosultap disodium has been shown to kill beetles in the crown (Ero 2016). This insecticide may be used for ornamental palms where CRB damage affects the aesthetic appearance, e.g. around tourist resorts. While systemic treatment with thiosultap disodium can be effective in coconut palms, it will leave residues in the nuts for an extended period (several months) after treatment. Systemic insecticides should therefore not be used on coconut palms unless nuts can be removed and destroyed to prevent consumption.

Some additional insecticide options for CRB have been described by CABI (2015), but their use is limited given the intermittent pattern of attack and the height of most palms making the crowns unreachable for application. As the target for protection is the base of the frond sheath where the beetle penetrates the petiole, granular formulations are an option to facilitate application on mature palms. Use of any insecticide should conform to the regulations of the relevant country and be applied with appropriate care to ensure worker safety.

4.4 Mechanical control

CRB adults in freshly damaged palms can be located and killed by workers using a wire hook in a process known as winkling. As the beetle bores into the palm petiole (section 2.3), it produces a large amount of cut fibrous material as it drills into the trunk (Figure 2.8). This will be evident in the axils of the fronds and will indicate the presence of a beetle. A wire can be inserted into the hole and, with skill, the beetle can be removed from the hole and killed. Winkling is more often used for juvenile oil and coconut palms where most CRB damage occurs in the crown and the fresh fibres produced by the beetle can be easily seen. In regions where other scarabs, such as *S. australis* or *O. centaurus*, may damage juvenile palms, this practice can be useful to collect adult beetles for identification and differentiation. Winkling is more difficult in mature palms as the worker must climb the palm to see the damage.

The use of netting to cover waste heaps and capture adult CRB entering or leaving is another example of mechanical control (Figure 4.2).

4.5 Internal biosecurity

If a CRB incursion is discovered early, it may be feasible to contain the pest to a restricted area. Containment will require cooperation of the affected communities because movement of planting materials that may carry CRB larvae or adults will need to be restricted. In addition, movement controls on sea and/or air travel will be needed. Strict controls were enacted in Vanuatu soon after the discovery of CRB on Efate (Paudel et al. 2023), and these controls have prevented widespread movement of CRB for several years. This containment was made possible by pre-existing legislation for internal biosecurity supported by ministerial orders and by the cooperation of shipping companies, who agreed to restrict loading/unloading activities to daylight hours on Efate. CRB adults fly to lights at night, so avoiding night activity at airports and seaports reduces the risk of adults landing on boats or aircraft and being transported to new locations.

Permanent containment to the initial invasion zone is unlikely to be feasible over longer time frames, as seen in Hawai'i (Coconut Rhinoceros Beetle Response – Hawai'i, nd), but if suitable legislation exists and cooperation can be negotiated, restricting movement will buy time (potentially up to several years) for biosecurity staff to develop response strategies and implement control measures. Limiting the spread of CRB will also reduce its impact at a national level.

4.6 Eradication

Eradication of any invasive species is challenging but is particularly difficult for cryptic or nocturnal pests like CRB. Only one successful eradication of CRB has been recorded so far, during the early invasion of this pest into Polynesia (Catley 1969). If eradication is to be attempted soon after the invasion is detected, the area infected by the initial CRB incursion must be small, and internal biosecurity measures must be mandated and strictly enforced to prevent further spread. Insecticides and sanitation are the primary tools for eradication, alongside pheromone trapping and palm damage surveys to monitor CRB activity. Care should be taken with the placement of pheromone traps during eradication attempts – traps should be placed within the infested zone but not in areas where there is no evidence of CRB presence (that is, no visible palm damage and no detection of beetles through manual searches). This caution with trap placement is necessary to

avoid attracting the beetles to new areas outside of the original infestation. Similar to containment through internal biosecurity, eradication may prove unsuccessful in the long run but attempting it can buy time for local authorities to build capability for long-term management of this pest.

4.7 Integrated pest management

Integrated pest management (IPM) is a broad-based approach that integrates practices for control of pests. The FAO defines IPM as "the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimise risks to human health and the environment. IPM emphasises the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms."

The Bioeconomy Science Institute (formerly AgResearch) and SPC recommend IPM to manage invasive CRB populations in the Pacific (Figure 4.7). This IPM plan relies primarily on three complementary control methods to suppress CRB populations successfully: classical biological control with OrNV, which typically infects adults and is transmitted amongst feeding aggregations in palm crowns; sanitation to remove breeding sites; and augmentative biological control with *M. majus*, which typically infects larvae and is most effective when applied to breeding sites that cannot be wholly removed by sanitation.

For coconut palms planted for subsistence, cultural uses, ornamental purposes, or in commercial plantations, IPM for CRB involves: (i) monitoring of palm damage to detect localised CRB outbreaks and check that control is successful; (ii) sanitation to remove organic waste, dead palms and other potential breeding sites; (iii) biological control with OrNV; and (iv) application of *M. majus* to breeding sites that cannot be removed.

Sanitation is an essential component of IPM for CRB that complements biological control (Huger 2005). Localised CRB outbreaks will occur when breeding sites are left unmanaged (e.g. after cyclones and tropical storms, when many palms may be toppled by high winds and large amounts of green waste are created). Historically, outbreaks of CRB often follow cyclones (Jackson and Marshall 2017). Many breeding sites are created during plantation renovation, when old palms are felled to make space for replanting. Larvae will develop in the decaying fronds, the trunk and even the root of the felled palm.

If resources are available to maintain pheromone traps for monitoring CRB, these can be incorporated into IPM programmes for coconut, particularly for commercial plantations. Occasionally, trap catches may be high enough to contribute to population suppression in coconut plantations (Bedford 2013), but this strategy is more relevant to oil palm (discussed below).

Insecticide treatments may be used to protect immature palms from CRB attack but are not recommended for established coconut palms as there is a high risk of insecticide translocation (movement within the palm), leaving harmful residues in the coconut. If a recent invasion of CRB is targeted for eradication, insecticides are almost certainly necessary for success. In this situation, expert advice is needed to determine the most appropriate choice of insecticide, to advise on the length of time residues will persist, and to ensure insecticide-contaminated coconuts are not harvested for human consumption. Use of personnel trained in the proper handling and application of insecticides is also strongly recommended.

For higher value crops, particularly oil palm, the same components are needed as for coconut: monitoring; sanitation; and biological control. More costly IPM components are recommended for oil palm because the crop's financial value makes greater investment in control worthwhile (reviewed by Bedford 2014). Thus, IPM for CRB in oil palm involves: (i) monitoring of beetle activity with pheromone traps and palm damage, particularly for young palms; (ii) sanitation to remove organic waste, particularly during plantation renewal when large amounts of waste are generated; (iii) biological control with OrNV plus application of *M. majus* biopesticides to breeding sites that cannot be removed, such as waste piles created during replanting; and (iv) insecticide treatments for young palms that are most sensitive to CRB damage. Note that pollinators of oil palm are vulnerable to insecticides, so applications should be scheduled carefully to avoid flowering. When oil palm plantations are renewed, complete clean-up of the organic waste is challenging. Application of *M. majus* to chipped trunks is recommended, prior to planting a cover crop to speed up decomposition.

Successful IPM is demonstrated by low levels of CRB damage on coconut and oil palms. In Pacific regions where historic CRB invasions are well controlled, occasional CRB outbreaks are associated with increased numbers of breeding sites, usually due to cyclones, plantation renewal, or poorly managed organic waste. Once effective sanitation is restored, damage declines again, although biosecurity authorities in these regions should be aware of the potential for invasion by different genetic subgroups of CRB. Progress is being made with the new wave of CRB invasions by working to restore biological control with OrNV, investing in capability development to use *Metarhizium* fungus effectively, and supporting affected communities to incorporate sanitation practices into their day-to-day crop management. Long term, investment in IPM for CRB will protect food security and livelihoods in the Pacific.

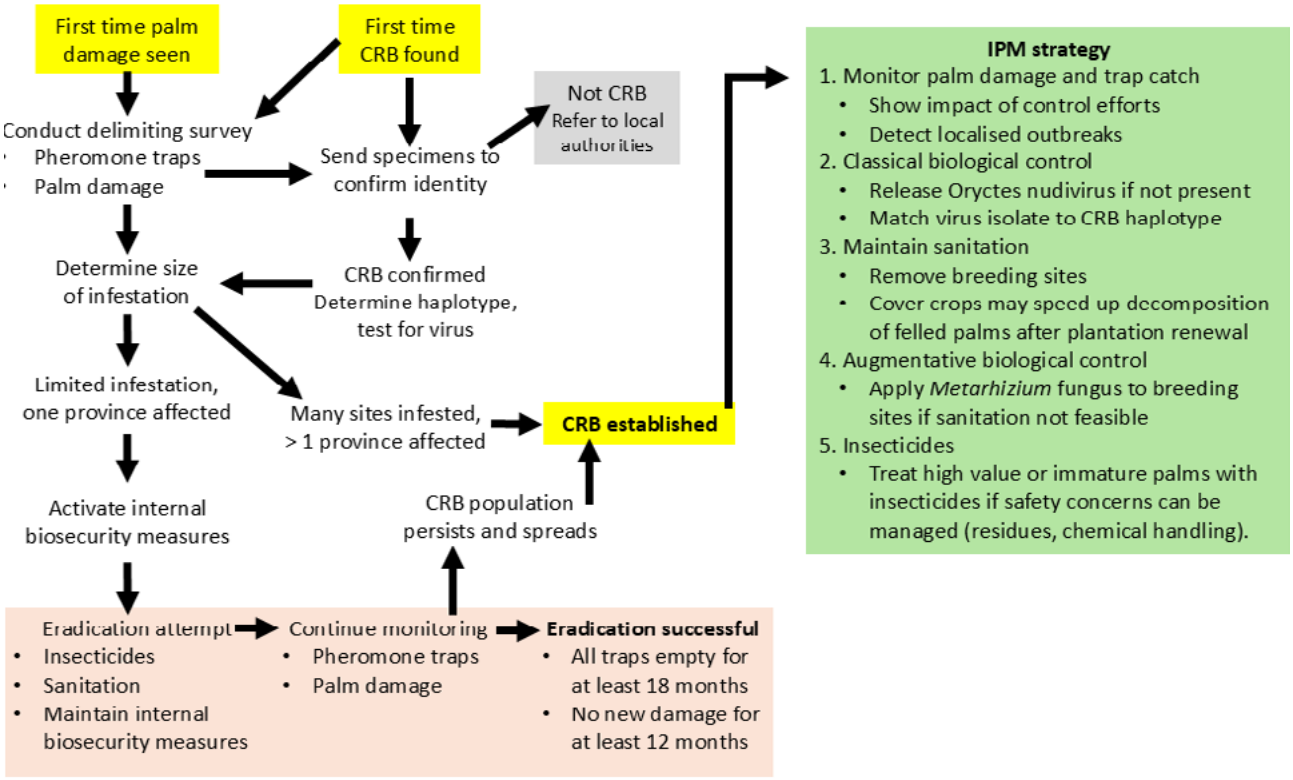


Figure 4.7 IPM decision tree for CRB control in its invasive range.

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Annex 1. Guidelines for field collection of live CRB adults and larvae

- CRB collection should be carried out at several sites during a delimiting survey.
- Use a separate container for each CRB collection site.
- Keep CRB adults separate from larvae if both are collected.
- Bring enough containers on the trip to ensure all collection sites can be sampled.
- Use sturdy plastic containers that have a screw top or clip top lid to prevent escape, about 500 mL in volume (~10 × 10 × 10 cm). These will each hold up to 10 individuals.
- Make small air holes in the tops of the containers.
- Handle CRB gently. Do not throw or drop them into the containers. Larvae are very sensitive to bruising.
- Add a small amount of breeding site compost (enough for the CRB to cover themselves).
- Only collect live CRB.
- Prioritise adult CRB for collection.
- Where this is not possible, collect larvae instead.
- During collection, label each container with a unique site name that can be traced back to the GPS location. Record the number of CRB collected, their life stage (adults or larvae), and for adults, the numbers of males and females, if possible.
- Store containers with live beetles out of direct sunlight so they do not overheat. Take care not to leave them in an enclosed unattended vehicle where temperatures can become very hot.
- Transport the beetles back to the biosecurity staff responsible for preservation of the tissue samples. These will be sent to a specialised laboratory for further analysis.
- Note that, while preservation of tissue in the field is theoretically possible, you need to have had the right training to ensure cross contamination between samples is avoided, and you need access to facilities on the field trip to ensure proper storage so the preserved tissue remains in good condition. Hence we recommend field collection of live CRB.

Collection of unusual CRB samples that may contain new pathogens

While conducting a survey, you may come across CRB adults or larvae that appear to be unusual and/or sites where mortality is higher than expected. If you do find any of these, take the steps outlined below.

- Record the location (GPS), take photos and, if possible, collect samples into individual tubes.
- Black larvae have been dead for some time. These are not as useful for collection.
- Record what you noticed that is unusual compared to healthy beetles you have observed, such as infected with fungus (fuzzy coating), odd colouration (red, bright white, light brown, etc.), lethargic, glossy or swollen larvae. For adults, fungal infection is most likely to be observed, but lethargic or other unusual symptoms may also to be seen.
- Note, fungus-infected insects (fuzzy coating) do not always indicate death by an insecticidal fungus. Following death, saprophytic (decay) fungi grow quickly and can look similar to some insecticidal fungi.
- Interesting sites tend to have multiple insects with similar symptoms. However, this is not always the case; it is possible to find unique specimens.
- Record the location and observations in your report so that the site can be revisited if it is deemed important.

Annex 2. Collection, preparation and shipment of CRB samples for DNA analysis

NOTE: Before preparing packages, contact the appropriate authority (e.g. SPC, local biosecurity officer) to confirm the likely species identification, documentation and packaging required for shipping specimens.

Sample collection

- Give each CRB specimen a unique reference number. Take photographs of each specimen.
- Record reference number and supporting information for each specimen on a data sheet.
- Supporting information includes: the name of the collector; the date it was collected; and a description of the location where it was collected and/or GPS coordinates.

Sample preparation for DNA analysis

- For each specimen, cut off both (2) hind legs and place them in a small (1–2 mL) leak-proof tube.
- Label the tube with the reference number.
- Add 1 mL monopropylene glycol (MPG) to the tube and close the lid.
- Store tubes in the freezer until they are ready to send to the specialist laboratory.

Packaging samples for shipment

- Place all tubes into a plastic Ziploc bag with some paper towels and seal the bag.
- Place the first bag of tubes into a second Ziploc bag and seal it.
- Place double bagged tubes into a crush-proof container (e.g. a high-quality food storage box) lined with paper towels and seal it.
- Wrap the container in bubble wrap or newspaper and place it in a strong cardboard box.
- Place required documents (outlined below) inside the box and then seal it with packing tape.

Documents and labels for shipment

- Inside the box, place a copy of the data sheet and a short description of the contents.
- Seal the box and attach labels, or place the box in a courier bag and attach labels to the bag.
- Make sure the labels include: (1) a short description of contents; (2) the sender's name and address; (3) the recipient's name and delivery address; and (4) a courier waybill or equivalent.
- Ship the package using a tracked courier service.
- Email the recipient the tracking number, the date the package was sent, and a copy of the datasheet.

Example CRB sample shipping label

Receiver (ship to): *[Name and address of specialist laboratory]*
Sender (shipped from): *[Your name and address]*
Description of contents:
For research purposes only. *[number of]* vials of dead, non-infectious coconut rhinoceros beetle *(Oryctes rhinoceros)* tissue preserved in monopropylene glycol (MPG). MPG is not classified as hazardous according to Schedules 1 to 6 of the Dangerous Goods Regulations. *[add country-specific regulations here]*

Annex 3. Production and field release of OrNV-infected adult CRB

1. Objectives and scope

This annex describes the methodology to use CRB larvae to mass produce OrNV, dosing of adult CRB using OrNV-infected larvae, field release of OrNV-infected CRB adults, and post-release monitoring. The aim is to conduct multiple releases of infected beetles at selected sites so that the chosen OrNV isolate becomes established within the CRB population and over time will suppress CRB and reduce palm damage.

2. Materials

2.1 Materials to prepare and dose CRB larvae

- Animal manure (e.g. from cattle or chickens)
- Partially composted sawdust (preferably from coconut, but other sources can be used, e.g. kapok wood)
- Sugar cane
- Plastic sheets
- Clean/sterile soft drink/beer cans
- Clean boxes to store CRB cans
- Plastic transfer pipettes
- Forceps and scissors
- CRB larvae (mid second (L2) to early/mid third (L3) instar)
- OrNV isolate selected for field release
- Bottled or sterile water
- Plastic sample containers, capacity 30–50 mL (big enough to hold one CRB L3 or two L2)
- A spoon or metal spatula (for crushing larvae)

2.2 Materials to conduct field releases

Items to take with you

- Clean/sterile soft drink/beer cans or small sample containers for CRB larvae
- Clean insulated box to transport CRB larvae in cans/containers on plane/boat
- Dosed CRB larvae (mid L2 to early/mid L3 instar)
- CRB larvae (mid L2 to early/mid L3 instar) that have not been dosed
- Tissue preservative: monopropylene glycol (MPG) (if available)
- Plastic transfer pipettes

Items needed at the release location

Check availability of essential supplies with local team prior to travel. If not available with local team, take these items with you.

- Pheromone sachets and traps to capture adult CRB locally
- Plastic container or bowl suitable for swimming adults
- Clean containers to hold adults after swimming and before release
- Clean containers to hold larvae after dosing (to maintain a source of OrNV)
- A spoon or metal spatula (for crushing larvae)
- Bottled or sterile water

3. Methodology

3.1 Before travel to the release location

Dosing (infecting) CRB larvae

- Collect second and third instar larvae from breeding sites and keep them individually in sterilised manure/sawdust mix. Hold for 7 days and dispose of any that do not feed or appear diseased.
- Dose larvae by putting two drops of the chosen OrNV isolate on pieces of sugar cane and providing these to the larvae for feeding. Repeat dosing on second day.
- Dose a minimum of 20 larvae for one field release.

Preparations for travel

- Handle larvae with care – they are precious. For travelling, it may help to add some sterile media into the larval containers, so they are cushioned from bumps and vibration.
- Consider taking extra undosed larvae (10–20) with you as spares – you can transfer OrNV infection to these to provide more material as you travel, particularly when you are travelling by boat for several days. If not used to produce OrNV, kill larvae before disposal.
- Contact local staff well in advance of travel date and ask them to start collecting adults for release and screening additional larvae prior to your arrival.

3.2 At the release location

When you arrive at the release location, use one of the dosing methods below to infect beetles ahead of their release. If only small numbers of adults (<20) are available for release, dosing on sugar cane is more efficient. For large numbers of beetles (>20), the swimming method will be more efficient.

Dosing beetles on sugar cane

- Use larvae that have been dosed twice with the chosen OrNV isolate and kept for 7 days to allow OrNV infection to develop. Larvae must be alive when virus is harvested.
- Take a 50 mL sample jar and one L3 or two L2 larvae. Chop heads off larvae, empty out internal fluid and macerate (crush) to release the virus solution inside the sample jar. When the body has emptied of fluid, fill the jar about half full with water to dilute the solution. Use bottled water or water that has been boiled then cooled. DO NOT use hot water.
- If MPG is available, preserve head capsule from each larva used to produce virus solution. Store in a freezer. These can be analysed later to confirm virus infection.
- Put two drops of the virus solution onto pieces of sugar cane and feed these to the beetles. Allow one piece of sugar cane for each beetle.
- Repeat dosing process the next day using freshly made virus solution, replacing sugar cane pieces if these have been eaten.
- On day 3, the beetles can be released at the selected site.

Dosing beetles by swimming in OrNV solution

- Use larvae that have been dosed twice with the chosen OrNV isolate and kept for 7 days to allow OrNV infection to develop. Larvae must be alive when virus is harvested.
- Take a plastic container or bowl. For a container about 20 × 10 × 10 cm, you will need two L3 or four L2 larvae. Chop heads off larvae, empty out internal fluid and macerate (crush) to release the virus solution inside the container or bowl. When the body has emptied of fluid, fill the container or bowl 2–3 cm deep with water to dilute the virus solution. Use bottled water or water that has been boiled then cooled. DO NOT use hot water.
- If MPG is available, preserve head capsule from each larva used to produce virus solution. Store in a freezer. These can be analysed later to confirm virus infection.
- Place beetles in the bowl and allow them to swim for 15–20 minutes. For a 20 × 10 × 10 cm container, you should be able to fit at least three beetles at a time. Beetles should be swimming without touching the bottom of the container/bowl. If they can touch the bottom of the container, add a little more water to increase the depth.
- Watch the beetles as they swim to make sure they do not climb out of the container or clamber over each other in the liquid so that they get above the water line.
- After swimming, take the beetles out and transfer to a clean container. Provide food (sugar cane). Add two drops of the virus solution to each piece of sugar cane to increase the dose.
- You can swim multiple batches of beetles through the same solution on the same day. Discard the solution when all batches have been swimming and provided with dosed food.
- Repeat the swimming process on day 2 using freshly made virus solution. Replace food pieces if eaten and add two drops of virus solution.
- Release adults on day 3.

Storage of leftover OrNV solution made from larvae

- If you have larvae available, it is best to use leftover solution to dose more larvae and generate fresh virus solution when needed.
- If you have solution left from the dosing process and you have access to a freezer, the leftover solution can be frozen for later use. This may be useful if the larval supply is limited or if you have temporarily run out of adults and need to store solution while you collect more. This stored solution is best used within 6 months.
- Only defrost frozen solution once, use it to dose adults and/or larvae then discard any leftovers.

Releasing adults after dosing

- Record dates of dosing, along with release information (date, location, number of beetles) using the example recording sheet below.
- Choose three release sites with clear evidence of CRB activity (medium and high palm damage) and somewhere for beetles to hide until ready to fly (on a palm trunk, under vegetation or similar).
- It is best to release late in the afternoon, not long before sundown. Do not release beetles close to a pheromone trap.
- Check the weather conditions and forecast (if available) before releases. If bad storms are expected that night, it is better to postpone the release for a day. Bad weather will increase the risk that the beetles fail to find a feeding site.
- Conduct three releases at each of the three sites. Rotate between sites (site 1, site 2, site 3, then begin again at site 1).
- When you do the first release at a new site, take photos or written notes of palm damage at the site. Assess ~50 palms at the site and record using the example recording sheet below.
- If possible, repeat releases at these sites or choose another three sites and again do three releases per site. This will depend on the size of the island where you are doing releases, how many larvae and adults can be collected, and when you run out of OrNV-infected larvae.
- Keep going as long as you have OrNV-infected larvae available – the more you release, the more likely the virus will establish. The target number for one release is 30–50 beetles; the minimum is 10 beetles.

3.3 Post-release monitoring

- 3 months after the last release of OrNV-dosed beetles, reassess palm damage at the release sites.
- Assess ~50 palms with photos and/or using the example recording sheet below.
- Compare observed damage with the pre-release assessment. If no improvement in palm damage is seen, wait 3 months and assess again.
- If resources and trained staff are available, collect adult CRB (using pheromone traps) and dissect to preserve gut samples for analysis.
- If resources are available, and no improvement in damage is seen, conduct more OrNV releases.

Example recording sheet for OrNV-infected beetle releases

Date first dose	Date second dose	Release date	Release site name	GPS lat/long	Beetles released	
					Number males	Number females

Example recording sheet for palm damage at OrNV-infected beetle release sites

Assess ~ 50 palms around each release site on the date of first release. Three months after last release, assess damage again. Use 5-point scale to assess damage. Take photos if possible.

Assessment date	Release site name	GPS lat/long	Number of palms in each damage category				
			None	***Low***	***Medium***	***High***	***Dead***

Annex 4. Application of *Metarhizium* sp. to control CRB in oil palm plantations

1. Objectives and scope

This annex describes the methodology to use the entomopathogenic fungus *Metarhizium* sp. (for example, *M. majus*) to control CRB in oil palm plantations. The fungus is applied as a liquid suspension and personal protection equipment must be worn to avoid inhalation of fungal spores. The extension officers, landowners and/or workers require suitable training before any application is made.

2. Methodology

- Define locations for fungus application. The application should be done soon after the palms have been felled and chipped but before sowing a cover crop. The fungus is applied to the rotten waste piles, comprising four rows of felled palms pushed into a single row of waste. If empty fruit bunches are used as mulch around newly planted seedlings, this mulch should also be treated with fungus.
- For spraying, prepare knapsack sprayers or a tractor-mounted mist blower that will ensure good coverage of the fungus suspension over the rotten waste piles.
- Wear protective equipment (gloves and face mask).
- Prepare the fungal suspension following the instructions provided by the manufacturer of the product or your own protocol developed for non-commercial fungus production. Ensure a minimum fungal concentration of 1×10^5 conidia/mL.
- Take the sprayer and the prepared fungal suspension to the site.
- Pour the fungal suspension into the sprayer tank that has been prefilled with a suitable volume of clean water (final volume depending on capacity but usually 1000 L).
- One side of the sprayer must be closed so the liquid is only sprayed from one side onto the heaps, avoiding product losses.
- Drive the tractor alongside the piles as you spray the fungus using an application rate of 1 L/m^2 and 1000 L/waste pile (Figure A4.1).
- Spray both sides of each pile until the liquid is finished.
- Prepare another 1000 L of the fungal spray, following the instructions above, and apply it to the next pile. Continue this process until all waste piles have been treated.
- The fungal treatment can be repeated after 6 months if decomposition is not finished.
- To evaluate the efficacy of the fungal treatment, each month assess CRB numbers in the piles by searching in several sections of each row. Monthly damage assessment of the replanted palms is also recommended.



Figure A4.1 Application of fungal suspension on piles using a tractor-mounted mist blower.

Annex 5. Application of *Metarhizium* sp. to CRB breeding sites

1. Objectives and scope

This annex describes the methodology to use the entomopathogenic fungus *Metarhizium majus* grown on cooked rice and applied to natural or artificial breeding sites to control CRB. The fungus should be used at locations where natural breeding sites with CRB populations are found or locations where palm damage suggests there is a CRB population in the area. The fungus must be applied using personal protection equipment and avoiding the inhalation of fungal spores. The extension officers, landowners and/or workers appointed to carry out the application require suitable training.

2. Methodology

2.1 Preapplication

- Define locations for potential *Metarhizium* application, for example, an area where sanitation has been carried out.
- Conduct a preapplication visit to identify the best site or sites for fungus application. Examples include:
    - places with natural breeding sites that need treatment (larvae present)
    - piles of chopped coconut trunks
    - piles of other crop waste.
- If no natural breeding sites are found, identify sites with damaged palms where an artificial breeding site (ABS; 1 × 1 m) can be created.
- Discuss with landowners where they would like *Metarhizium* to be applied, and if required, where the ABS should be created.
- Once agreement is reached with the landowners, if an ABS is needed, it should be set up during the preapplication visit using local material available at the site (if time permits).
- As part of the preapplication visit, record the exact locations selected for application, the type of breeding site (natural or artificial), and take pictures of the site and the palms with GPS (use Kobo Toolbox, camera, or phone with GPS). If GPS is not possible, record a description of the site. See below for example recording sheet.

2.2 *Metarhizium* application

- No more than a week after the preapplication visit, decide on the day for application considering the weather conditions (avoid heavy rain or storms).

- On the day of the application take the *Metarhizium*-sporulated rice (from your local production facility) required for the breeding sites to be treated.
- Transport the *Metarhizium*-sporulated rice in an insulated container (chilly bin, drinks cooler, polystyrene box).
- Bring protective equipment (gloves, face mask) for each person doing the application and scissors if needed to open the fungus bags.
- Go to the chosen breeding site.
- Wear protective equipment (gloves, face mask).



- Take out the fungus from the chiller.
- Sprinkle the sporulated rice over the surface of the breeding material (at least 200 g of sporulated rice per breeding site).
- If the breeding material is suitable (soft and partially decomposed), mix the sporulated rice by hand.
- If the breeding material is fresher (large pieces of trunks or similar), turn over the pieces to protect the fungus from sunlight.
- After mixing or turning over, cover the breeding site with fresh leaves or other organic material.
- If there is more than one breeding site at the same location, repeat the process for each site.
- Record the date of application, the batch number of the fungus bags applied to each site and how many sites were treated, using a recording sheet based on the example below.

2.3 Assessment and reapplication of *Metarhizium*

- After 3 months, check the weather conditions and confirm with landowners the date to carry out the visit.
- On the day of the visit take fresh *Metarhizium*-sporulated rice to be applied on breeding site.
- Transport the fungus in an insulated container (chilly bin, drinks cooler, polystyrene box).
- Bring protective equipment (gloves, face mask) for each person doing the application and scissors if needed to open the fungus bags.
- Go to the chosen breeding site.
- Wear protective equipment (gloves, face mask).



- Check inside the breeding site and assess the level of decay. If there is still breeding material present or you find CRB grubs, then apply the fungus (at least 200 g per breeding site), following the instructions described for the first application.
- After mixing or turning over, cover the breeding site with fresh leaves or other organic material.
- If there is more than one breeding site at the same location, check each one and reapply if needed.
- Record the date of application, the batch number of the fungus bags applied in each site, and how many sites were treated, and take pictures of the palms.
- If all the breeding sites are decayed, discuss with landowners if they want new ABS established and treated.
- Visit the sites again after 3 months and repeat the assessment, reapplication of fungus or establishment of new ABS if needed.

3. Example recording sheets for fungus application

Fungus preapplication data sheet

Date of visit		Officer's name	
Location	Village name		
	Island		
	Province		
Number of breeding sites		GPS for each breeding site	
Photos of breeding sites taken (Y/N)		Photos of palms taken (Y/N)	

Fungus application data sheet

Date of visit		Officer's name			
Location	Village name				
	Island				
	Province				
Number of breeding sites treated		Fungus batch number		Quantity of fungus/breeding site	

Fungus assessment and reapplication data sheet

Date of visit		Officer's name	
Location	Village name		
	Island		
	Province		
Number of breeding sites treated again		Number of breeding sites completely decayed	
Fungus batch number		Quantity of fungus/ breeding site	
Photos of breeding sites taken (Y/N)		Photos of palms taken (Y/N)	

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