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

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Introduction

This manual has been developed to support trainers in building the capacity of biosecurity and extension workers for the control and management of coconut rhinoceros beetles (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 control and manage this pest. Publication of this manual is timely as the Pacific region is challenged by the invasion of a new CRB biotype, the CRB-G, and there remains a need to regain control of the established biotype CRB-S, 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.

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 the positive reviews from Bedford (1980, 2013a), Godshen (2015), Huger (2005) or the numerous works of Zelazny and others, from which this manual draws extensively. Summaries on CRB are also provided by CABI (www.cabi.org/isc/datasheet/37974, accessed 3 September 2019) and PESTNET (http://www.pestnet.org/fact_sheets/coconut_rhinoceros_beetle__oryctes_108.htm, accessed 3 September 2019). 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 is provided in this manual 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.

The manual is divided into three 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, recognition and assessment of the damage it causes, as well as methods for collection and handling of the pest after it has been identified. Relevant contacts are also provided to facilitate access to expert assistance, where needed, and methods are outlined for further diagnostics. Obtaining this information and recording it in a systematic way is essential to: a) conduct delimitation surveys; b) measure the severity of attack; and c) provide a baseline to monitor the effectiveness of control actions. The third section covers control actions for CRB in the Pacific and draws on the experiences of our colleagues in the CRB Action Task Force, particularly those working in Guam, Solomon Islands and Papua New Guinea (PNG) to control the invasive CRB-G biotype.

The emergence of CRB-G as an invasive pest underpins the need for a revision of data and a revitalisation of the IPM system for CRB to protect coconut and oil palm production. This issue prompted a revision of CRB data and production of this manual.

Section 1. Coconut rhinoceros beetle in the Pacific

1.1 Review

For over a century, Pacific Island authorities and states have been confronted by an invasive insect from Asia, CRB (*Oryctes rhinoceros* L.). The CRB was inadvertently introduced into Upolu, Samoa, 1909 in rubber seedling plants in pots from Ceylon (now Sri Lanka) and, then, established itself on the island, multiplied and spread rapidly, causing extensive damage to coconut palms (*Cocos nucifera*) and requiring measures for its management. The initial invasion, described by Bedford (1980), spread throughout Samoa and American Samoa to Niuatoputapu Island (Tonga) (where it was eradicated) and onward to Wallis Island, Tongatapu (Tonga), Tokelau Islands and Fiji, where it had covered most of the inhabited islands by 1953. Interestingly, DNA analysis of specimens from the main islands indicates that they belong to the same biotype (Marshall, et al. 2017), suggesting that the population expansion was indeed caused by the original introduction of the 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, Catley 1969). 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 this group as separate 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, the population was 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. The CRB had a similar effect on other islands, where controls were omitted or ineffective; losses of 50 per cent or more of the standing palms were reported in some areas (Gressitt 1953). Scientists recognised that the CRB invasion on 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 an 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 Food and Agriculture Organization (FAO), and SPC, funded a series of projects from the 1960s to 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 Likely CRB biosecurity high-risk pathways into a country

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 in Samoa where it rapidly spread and, subsequently, was introduced in the other infested islands where it infected the beetle populations, spread and reduced palm damage (Bedford 1976). The history of virus discovery and use is described by Huger (2005) and Jackson (2009). The virus has been characterised by gene sequencing as the *Oryctes rhinoceros Nudivirus (OrNV)* (Wang et al. 2008). In recent years, use of DNA PCR detection methods has shown that the virus 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 2013b). The virus release programme was so successful that no further spread of CRB from the virus-infected populations was reported. While the virus weakened the infected beetle populations and reduced damage, however, 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. (Further information is provided below.)

In 2007, a new and highly damaging outbreak of CRB was reported from the island of Guam. Surprisingly, the beetles were unaffected by the existing biocontrol strains of *OrNV* and other control measures were insufficient to prevent the beetle from spreading around the whole island. The attack on Guam marked the start of a new wave of damaging invasions of CRB reported from Hawaii (Honolulu), PNG (Port Moresby), and the Solomon Islands (Honiara). These new invasions were all found to be caused by a distinct biotype of the beetle, CRB-G (Marshall et al. 2017). CRB-G is defined by genotype and as a distinct biotype that is resistant/tolerant to biocontrol strains of the *OrNV*. The CRB-S biotype is defined as beetles susceptible to *OrNV*. The CRB-G and CRB-S biotypes are each comprised of several haplotypes (genetically distinct subgroups within a species) reflecting their geographic origin (Marshall et al. 2017). Genetic analysis suggests that the CRB-G outbreaks have been mediated through human transport from a source in the Asian Northwest Pacific (Reil et al. 2018).

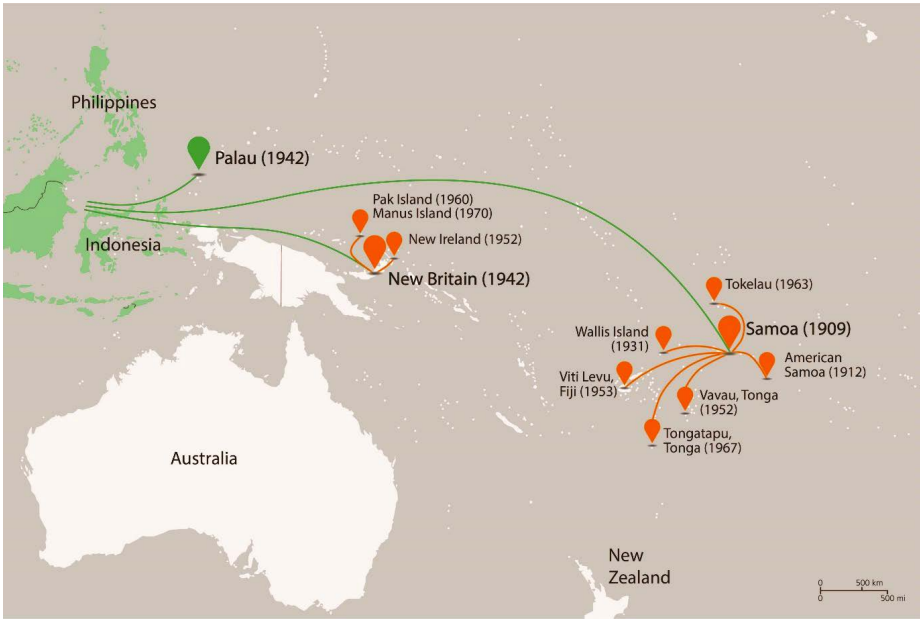

Figure 1.1 Historic distribution of CRB in the Pacific (Redrawn from Catley 1969)

Key: Green indicates the native range of CRB; and orange indicates an invasion by CRB-S.
The current distribution of CRB is presented in an online interactive map showing reports of CRB from the endemic zone (green symbols), the original invasion zone (orange symbols) and recent invasions by CRB-G (red symbols) (Moore 2018, http://aubreymoore.github.io/crbdist/mymap.html accessed 5 July 2019). A snapshot of this map is shown below (Figure 1.2).

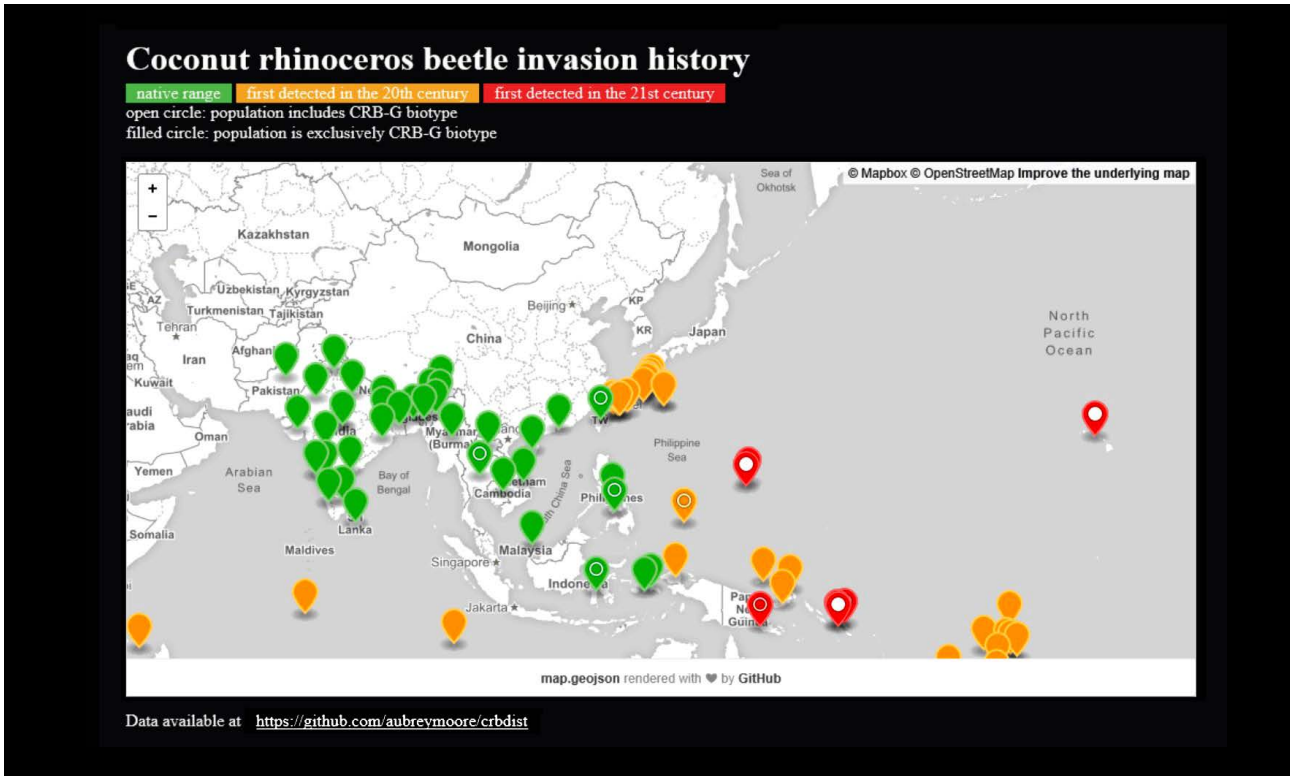

Figure 1.2 Native and invaded geographic range of CRB, including the recent invasion of CRB-G (courtesy of Aubrey Moore, University of Guam)

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Section 2. Diagnostics: Pest and symptom recognition

2.1 Beetle life cycle and recognition

The CRB belongs to the sub-family Dynastinae of the large Scarabaeidae family of beetles. The Dynastinae include a wide range of long-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 undergoes the typical scarab beetle life cycle, growing from egg to adult through three larval stages (instars) before transforming through pupation into a new adult (Figure 2.1).

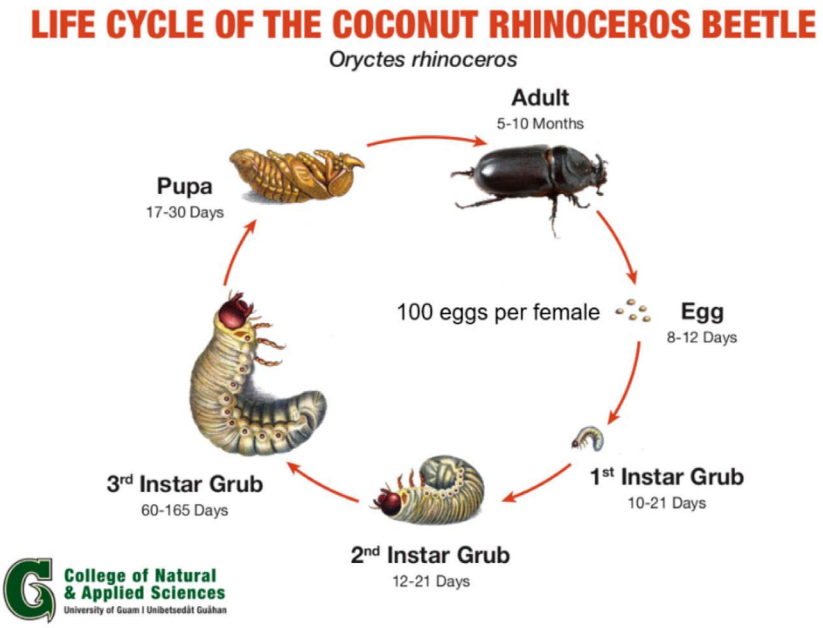

Figure 2.1 Life cycle of the CRB (University of Guam)

The duration of the life cycle will depend on environmental conditions, but the time from egg to adult can be as little as five 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 x 4.0 millimetres) and produce the first instar larvae in 8–12 days. Larvae are C-shaped, whitish with a reddish-brown head capsule with sclerotised mouthparts, a darkened area visible in the posterior abdomen « fermentation chamber » indicating the internal modification of the gut (Figure 2.2).

Figure 2.2 Larva of CRB

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 millimetre in length and about 10 grams in weight (Figure 2.2). Prior to pupation, the larvae stop feeding and their bodies change to a creamy colour and shrink slightly. The pre-pupal larva forms a cell and differentiates into the pupal stage which lasts for approximately three weeks before metamorphosising 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 millimetres in length and 4–12 grams 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).

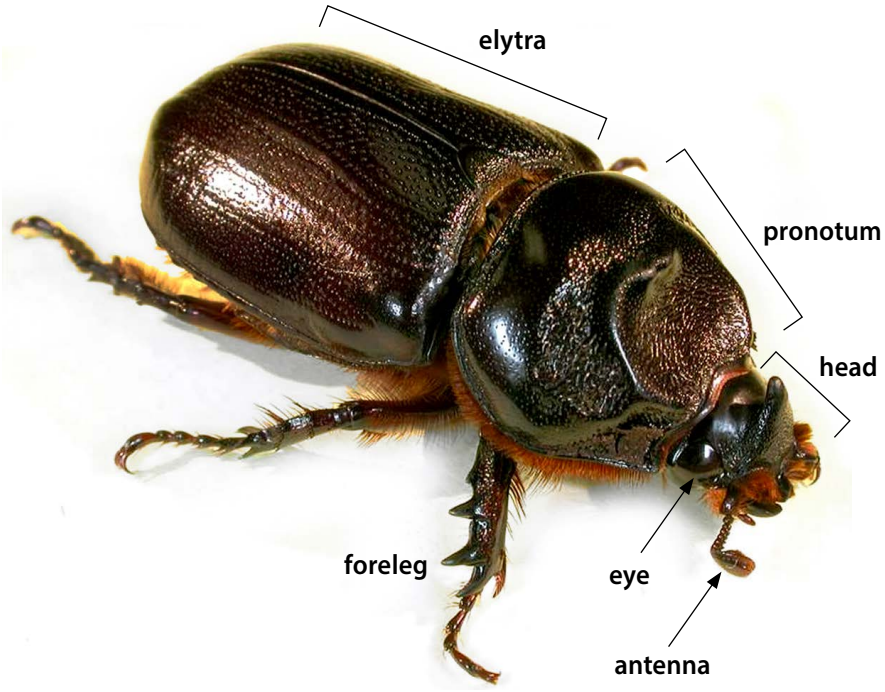

Figure 2.3 Adult CRB

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 laminate antennae, typical of the Scarabaeidae, extend laterally concealing the strong mandibles which are used for grasping. 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).

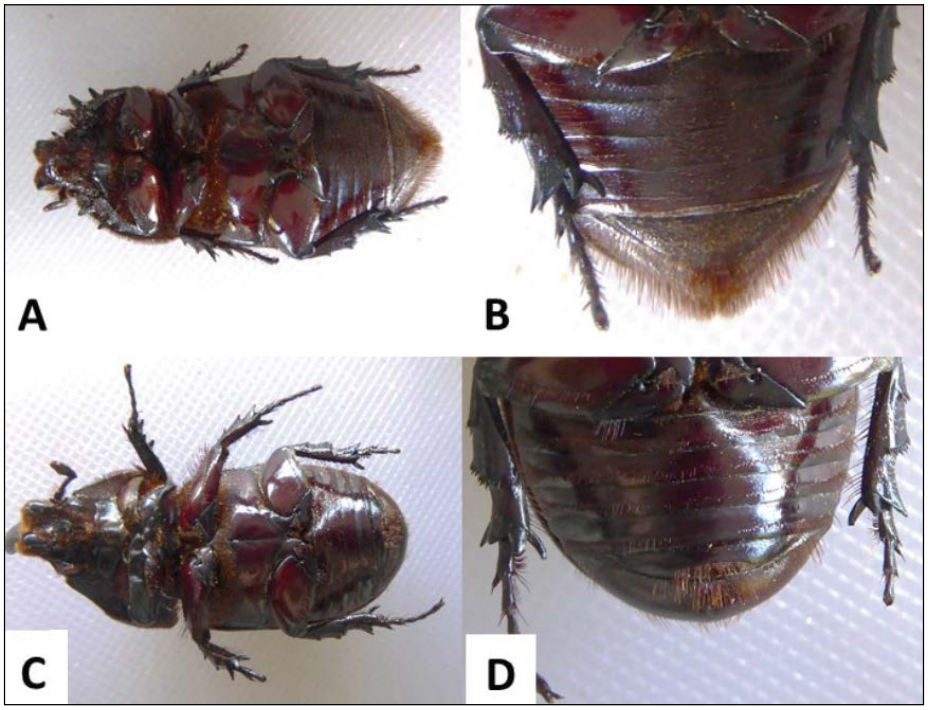

Figure 2.4 The pygidium (terminal section of the abdomen) of the female CRB is covered with fine hairs (A&B) while the posterior of the male is shiny (C&D). On dissection, the abdomen of the female may contain eggs while the male has a large chitinous aedeagus (penis) in the abdominal cavity.

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 (Cetoninae), 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). Melanesian rhinoceros beetle *(Scapanes australis)* and brown rhinoceros beetle (*Xylotrupes gideon)* occupy similar ecological niches and some stages, especially small larvae, are difficult to differentiate from CRB. Descriptions of co-habiting species and keys for identification are provided by Bedford (1974, 1976) and Beaudoin-Ollivier et al. (2000). To confirm identity, specimens should be sent to Plant Health Laboratory of SPC's Land Resources Division.

To date, no morphological characteristics have been identified which can be used to differentiate biotypes of CRB. Therefore, genetic methods must be used. Tissue samples must be collected from fresh CRB specimens. Isolation of DNA and biotype identification require specialised facilities. Please contact the SPC Entomologist, mentioned above, regarding shipment of specimens for genetic analysis (Annex 4).

2.2 Sample collection and handling

CRB adults or larvae are required for identification, ecological studies or 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 a "chilly bin" (insulated picnic cooler) or frozen blocks to keep it cool as well as several containers or individual tubes for the insects. (Refer to Figure 2.5. Note also that guidelines for sample collection are provided in Annex 3.)

Figure 2.5 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.

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 the compost, the species of the palm, and the level of damage and density of the 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 is maintained in a central database.

The precise manner of the 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) in order 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 to place them in individual tubes (25 millilitre 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.

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, please 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 2.6). The damage is caused by adult beetles flying to the palm crown and walking down the frond axil until they are lodged between the open frond and the growing spear (Figure 2.7). Securely positioned, they will bore through the spear, cutting leaflets in the compacted un-emerged frond before they reach the soft tissue of the young under-developed fronds. In the most severe cases, beetles will feed on soft leaf tissues (Figure 2.8) 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 2.9.

Figure 2.6 Coconut palm showing distinctive v-shaped notches in the fronds caused by CRB adult feeding

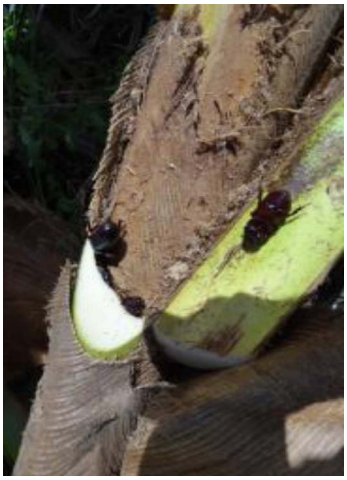

Figure 2.7 CRB adults in the axil between the emerging frond and the spear

Figure 2.8 CRB adults feeding in the soft, core tissue of the palm

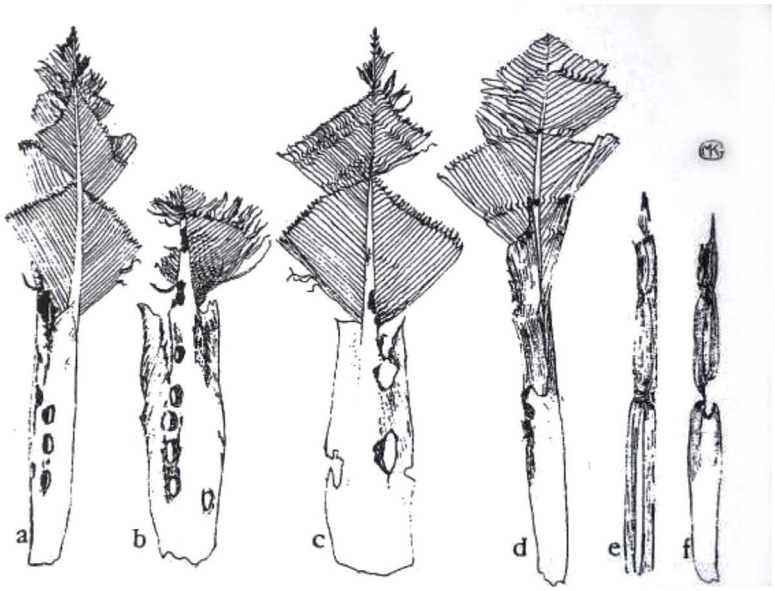

Figure 2.9 Damage to the central growing point of the palm (Gressitt 1953)

2.3 Damage assessment

a) Damage assessment in the delimitation survey

Damage assessment should be a part of the delimitation survey. (For further information, see Section 2.5) 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 kilometres until no further damage is noted. The results should be discussed with the local community and a reporting system will need to be established in order to monitor potential spread.

b) Quantifying palm damage

Quantifying damage from CRB to coconut or oil palm allows sites to be compared. Damage assessment methods can be simple, used only to define the location and intensity of the damage, or they can be more sophisticated, used to monitor changes over time.

c) Simple rapid damage assessment (RDA) by counting

Rapid damage assessment (RDA) protocol: Within the assessment area, select a site and mark the position by GPS or a map. At each site, assess 30 to 100 palms individually for signs of CRB damage and record whether or not damage has been identified (Y = damaged or N = undamaged). Calculate the percentage (%) of palms damaged as a simple damage index (between 0 and 100). (See Table 2.1 for an example.) Digital photographs should be taken to confirm the visual survey, or a set of photographs can be taken from the site and assessed later. Once calculated, this damage index can be correlated with other factors (e.g. geographical location, plantation management, biocontrol, etc.) and analysed, where necessary.

Table 2.1 Calculation of the damage index using the rapid damage assessment protocol for coconut palms

Site	Palms observed	Palms damaged	Damage index
A	30	6	20
B	50	35	70
C	100	28	28
D	37	26	70
E	45	42	93
F	66	13	20

d) Quantified damage assessment (QDA)

To gain further information, a quantified damage assessment (QDA) can be used. This is most appropriate where there is a moderate or high proportion of palms damaged and QDA can be used to estimate the level of impact of CRB. QDA can follow on from RDA (in the field or from photos). The level of damage to the crown can be assessed on a 0 to 5 scale. (See Table 2.2.)

Assessments can be made on a recoding sheet in the field or from photographs of the site. In either case, it is important that a clear view can be obtained of the individual palm crowns. Particular attention should be given to ensure that assessment of individual palms is not repeated (double counting) when moving around.

QDAs are easier to conduct on scattered palms in damaged plantations or in villages where damage may be underestimated in dense plantations.

Multiple photographs or strip photographs can be used to quantify the damage at a site. Individual palms from the photo(s) (Figure 2.10) can be graded according to the QDA scale (Table 2.2) and, subsequently, summed into groups and the leaf area loss estimated.

Figure 2.10 Coconut palms from a badly damaged site graded on the 1–5 damage scale (Table 2.2)

From the badly damaged site of Figure 2.10, a 65 per cent foliage loss can be estimated (Figure 2.11). Young (1975) established a relationship between leaf area loss and yield; this indicates little yield from the pictured site, which is in line with practical expectations.

Table 2.2 Grading scale for damage to coconut palm fronds caused by CRB

GRADE AND DESCRIPTION				
1	**2**	**3**	**4**	**5**
				
No CRB damage evident	**Light**: Light damage Notching or tip damage. <20% foliar loss	**Medium**: Multiple fronds affected Notching and breakage. 20-50% frond loss	**High**: Multiple fronds affected Notching and breakage. >50% frond loss	**Non-recoverable**: Palm dead or growing point destroyed

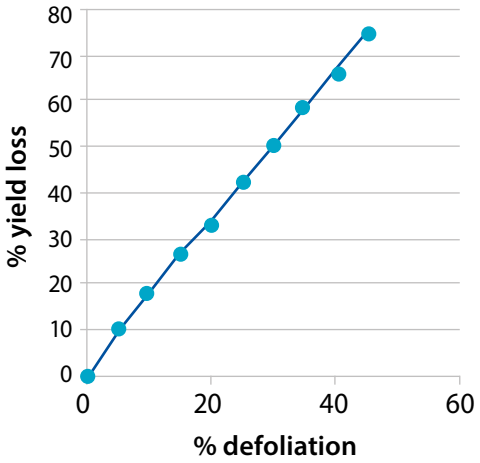

Figure 2.11 Relationship between leaf area loss and yield (Young 1975)

e) Damage assessment as a biological clock

A healthy coconut palm growing in favourable environmental and nutritional conditions will produce fronds at approximate monthly intervals and hold approximately 20–25 fronds before they senesce and drop. Additionally, from formation in the growing point to emergence takes approximately three 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 2.12). Estimating the age of first attack may provide clues to the arrival of a CRB incursion. It also indicates success or failure of a CRB control action.

f) Monitoring new growth for change

Recording damage to the topmost four fronds gives an estimate of feeding and damage that has occurred in the previous six months. This measure can be used to assess the effectiveness of control actions (trapping, sanitation) against the beetle over a short time period and can be used for activity monitoring.

Figure 2.12 Estimated age in months of fronds (from formation) on a mature coconut palm

2.4 Delimitation and baseline surveys

In an advent of an 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.

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

1. Establish exactly how and when the pest reached the area.
2. Monitor the speed of the pest's dispersal.
3. Map boundaries and estimate the size of both the area already infested and possible areas into which the pest could spread.
4. Assess the area currently covered by the host plants within the concerned sites.
5. Assess the financial loss and social damage caused by the pest if it spreads to the whole endangered area.
6. Identify plants, plant products or other articles, whose movement out of the infested area need to be regulated in order to contain the pest.
7. Identify owner(s) of the plants, plant products or other articles.
8. Identify how these plants or other articles could spread further (wind, human transport) e.g. boat, aircraft, private and public vehicles)].
9. Assess the possibilities of stopping the pest from spreading further.
10. Assess the feasibility, cost and possible problems of containing, eradicating and managing the pest outbreaks.
11. Identify how and where infested plants and/or products could be treated or disposed.
12. Take pictures of the pest, symptoms, affected plants and areas.
13. Inform local authorities, extension officers and producers about the host crops.
14. Recommend local staff who would need to be engaged in further actions.

As an example, Annex 1 provides the procedures used for the delimiting survey implemented in the Solomon Islands after the invasion of CRB-G.

Once an invasion of CRB is confirmed, an Emergency Response Plan should be developed, based on the Biosecurity Incident Management System (BIMS) framework provided in Annex 2.

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Section 3. Control actions for CRB

CRB is endemic 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. 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 senile palms for establishment of new plantations (Figure 3.1). In these conditions, female beetles will 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 and emergence of particularly high numbers in the second generation. These numerous emergent adults will cause significant damage to the nearby palms, even killing many, which leads to further substrate availability and an ongoing problem from the beetles.

Figure 3.1 A) High numbers of CRB larvae removed from a section of palm trunk
B) Heavy damage to a coconut plantation near Honiara, Solomon Islands

3.1 Sanitation

Sanitation is a process to remove organic matter sources and is especially important to prevent establishment of new populations and to limit the 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 can be removed, or at least reduced. Sanitation methods are reviewed in Godshen (2015) and the sanitation programme carried out on Guam has been described by Smith and Moore (2008). Where sanitation is part of the response to a recent invasion by CRB, it is important to keep thorough records of clean-up activities. In any programme to control CRB, a sanitation plan should be developed and carried out, as outlined below.

i. Removal of standing dead palms

CRB adults are attracted to standing dead 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 strength of their mandibles, they can penetrate further down the trunk leaving a

column of frass and cut fibres for the early instars. Dead standing palms should be felled, cut into pieces and burnt or buried to remove potential breeding sites. 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). The organic matter should be removed where possible.

ii. Disposal of dead felled palms

Mature palm trees will fall after being weakened by fungal diseases (*Ganoderma*), after strong winds during cyclones or after the felling of senile palms prior to replanting. The dead palms on the ground should be cut up into manageable lengths prior to disposal by burning or burying. For oil palm plantation renovation, cutting the trunk into small lengths to accelerate breakdown is recommended.

iii. Covering of palm stumps

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 and make it less attractive to the flying beetles. The legumes *Mucuna* spp. and *Pueraria javanica* are ground cover plants that are commonly used, as they will add nitrogen to the soil and cover the decaying trunks. The same principles should be used by villagers to manage damaged palms. Management of ground cover in Asia is reviewed by Sahid and Weng (2000).

iv. 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 fruit bunches are particularly susceptible. Sawdust from sawmills that process palm timber is also a favourable resource for beetle development. General compost, farmyard manure and even organic garbage can provide sites for development of the larvae. The first step in reducing the threat of beetles emerging from composts 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. (For further information, please see below.)

3.2 Trapping

CRB trapping can be used for different purposes. These include surveillance for early detection, monitoring a population over time, or for mass trapping 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 held inside. Attractants can be used to encourage the insect to enter the trap.

One of the first traps to be developed was the Hoyt trap made from a metal can set on top of a coconut trunk (Fig 3.2 A, Hoyt 1963). The can was capped with a round of coconut stem with a hole in the centre large enough for a beetle to enter. The trap system was used extensively (e.g. Bedford 1975) and functioned because the standing, decaying coconut stem was attractive to the beetle. Another system was a split log trap (Bedford 1976b). Sections of split coconut log were laid on the ground and beetles would conceal themselves below the log sections. Design and utility of traps changed with the discovery of ethyl crysanthemumate which can be used as an attractant. This was rapidly superseded by ethyl4-methyloctanoate (E4-MO), the male-produced aggregation pheromone of CRB, which could be synthesised. 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).

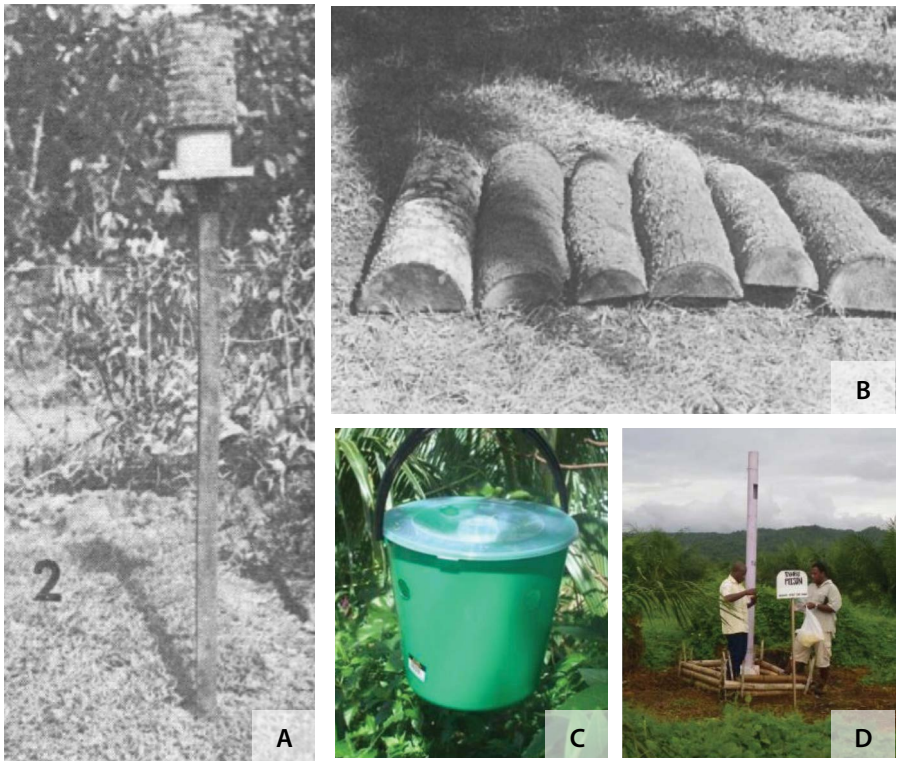

Figure 3.2 CRB traps: A) Hoyt trap (Bedford 1973); B) Log trap (Bedford 1976b); C) Bucket trap (SPC); D) Pipe trap (PNGOPRA)

Traps for surveillance need to be robust, inexpensive, attractive to beetles, difficult to exit and simple to service. Bucket traps, often with vanes, have been used in surveillance trapping in Guam and Hawaii where thousands of traps have been distributed and monitored 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.

Construction of a bucket trap

The pheromone bucket trap can be constructed from a plastic bucket with a lid. Two large holes (with a diameter of 2.8 centimetres) and 2 small holes (in the centre) should be made in the lid as illustrated below. The holes can be cut using a hot wire or hot rod.

The pheromone sachet should be opened and attached with a wire under the bucket lid. The operator should ensure the sachet is not damaged and that it is placed in an upright position inside the bucket.

The lid should be placed on the bucket (serving as the pheromone trap). In the field, the pheromone trap should be placed on a strong branch with the bucket hanging upright (Prasad S and Lal S, 2006). For diagnostic purposes, such as DNA analysis, beetles should be removed at least once per week and stored individually in plastic containers. If used for monitoring adult beetle numbers, the bucket should be emptied at three-week intervals and the collected beetles destroyed. Pheromone sachets need to be replaced every 6–8 weeks (average), given the tropic heat and evaporation levels.

Stages in the construction of a bucket trap. Photos: SPC

A more costly alternative to the bucket trap is the pipe trap (Figure 3.2D), which was developed by the oil palm industry in PNG and has been widely used. It is made from a two-metre section of PVC piping secured to an iron bar above a collection tray. Two "windows" are cut into the pipe near the top, which aid entry of the beetles before they fall into the collection chamber. Pipe traps are fixed in surveillance sites and suitable for long-term monitoring.

Sachets of E4-MO can be placed in either bucket or pipe traps as a lure for the beetles. Traps should be placed in a location that is attractive to 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 (usually every 7–14 days) with beetles differentiated into males and females and numbers recorded. Sachets of lure should be replaced when they appear to have dried out. This depends on weather conditions, but sachets usually last 1–4 months.

The E4-MO pheromone is an aggregation pheromone that is attractive to both male and female beetles. The pheromone assists the general orientation of the beetles. However, they are not able to identify the precise location and many will arrive in the vicinity of the trap without entering it. This can result in higher damage to palms close to the trap when compared with the average of the whole plantation.

In the office, trap catches should be analysed, preferably mapped and summarised. The trap catch should be converted to beetles trapped per day, as this figure will be comparable between sites and dates for the same type of trap. An example is presented below, with daily trap catch mapped with time (Figure 3.3).

Trap information should be complemented with regular photographic assessment of palms around the sample area and satellite images may be used to assist monitoring. Dated satellite images from Google Earth PRO can also be used to provide an indication of changes in damage over time.

Trapping will remove insects from the population and can contribute to pest and damage reduction and even eradication. Bucket traps baited with pheromone have been reported to reduce CRB populations in Malaysia (Chung 1997) and the related *O. monoceros* in West Africa (Allou 2006). However, bucket and pipe traps are not sufficient for high-density, invasive populations.

	Trap Collection					
Trap collection date	Male	Female	Dead	Total	Number of days	Beetles per 14 days
3/09/16	1	0	0	1		
17/09/16	1	2	0	3	14	3.0
1/10/16	0	2	0	2	14	2.0
15/10/16	0	4	2	6	14	6.0
2/11/16	4	2	0	6	18	4.7
23/02/17	1	1	1	3		
11/03/17	2	4	0	6	16	5.3
15/04/17	4	8	1	13	35	5.2
16/05/17	0	0	0	0	30	0.0
3/06/17	0	0	0	0	18	0.0
16/06/17	0	0	0	0	13	0.0
1/07/17	0	0	0	0	15	0.0
15/07/17	0	0	0	0	14	0.0
29/07/17	0	0	0	0	14	0.0
12/08/17	1	0	0	1	14	1.0
					Overall Average/ 14 days	
Total	14	23	4	41	2016	3.9
Average	0.9	1.5	0.3		2017	1.3
%	34%	56%	10%			

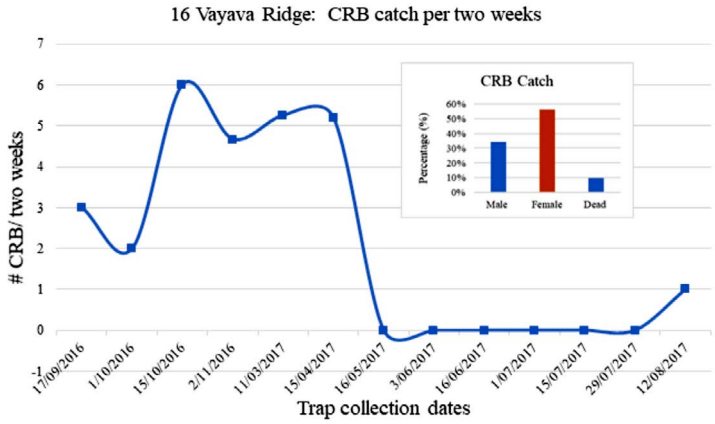

Figure 3.3 Pheromone trap collection data sheet
(Example modified from data provided by Biosecurity Solomon Islands (BSI), Honiara, Solomon Islands)

Improving trap efficiency has been a goal of the research team at the University of Guam where a range of innovative trap designs have been developed (Moore et al. 2014; Iriate et al. 2015). Bucket trap catches have been improved by expanding the size (a barrel trap), and adding organic matter to the trap and a small LED light. In an alternative approach, fish nets made from Tekken netting have been used (Figure 3.4). These act like a fishing gill net, catching beetles as they try to move through it. In Guam, covering heaps of organic waste with Tekken netting has been successful as it catches fresh adults as they emerge from organic waste, where they have developed, as well as beetles that are returning to the organic matter heaps to lay eggs. Netting can also be used in simple traps baited with pheromone on fences or by looping around the palm trunk to entangle the adult beetles as they move to the palm (Moore et al. 2014).

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.

Tekken net covering a large pile in fresh organic material.

CRB caught in Tekken netting

Figure 3.4 Use of Tekken netting traps to capture CRB (Iriarte et al. 2015)

Trapping has been used successfully for both monitoring and control. The Hoyt trap was used to monitor the decline of beetle numbers over three years, which was associated with a loss of organic matter for larval feeding (Bedford 1975). Pheromone traps baited with E4-MO can be placed at one trap per 10 hectares to monitor populations in order to establish control action thresholds, or they can be placed at high densities, one per two hectares, in order to reduce CRB populations in plantations (Chung 1997). With improvements in trap design, Moore et al. (2014) estimated the capture of 33 per cent of a CRB population, which would have a significant impact on the population especially if combined with other measures. Tekken netting tied around the palm through the leaf axils seems particularly effective in protecting young ornamental palms from attack as it intercepts the beetles as they move towards the feeding site.

3.3 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 2.5), it produces an excessive amount of cut fibrous material as it drills into the trunk (Fig. 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 mostly used in oil palm where most damage occurs in the crown of young palms and the fresh fibres produced by the beetle can be easily seen. Winkling in coconut palms is more difficult as the worker must climb the palm to see the damage.

3.4 Chemical control

Chemical control measures are most effective on young palms against CRB. Chemical pesticides are used to prevent the adult beetle from damaging the spear and growing point. The insecticide, cypermethrin, is recommended to protect young oil palm replants in CRB-infested areas but only until the palm starts fruiting as the insecticides can damage the beneficial pollinating weevil (*Elaeidobius kamerunicus*) (Ismael et al. 2009). Moore (2013) also tested use of cypermethrin for control of CRB in coconut palms in Guam and found it effective when applied to young damaged palms. Trunk injection with Thiosultap disodium (Ero 2016) has also been shown to kill beetles in the crown of mature oil palms (Ero pers. comm.). Some insecticide options for CRB have been described by CABI (www.cabi.org/isc/datasheet/37974); however, their use is limited given the intermittent pattern of attack and the growth of the palms making the crowns unreachable. 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. Use of any insecticide should conform to the registration regulations of the country of use and be applied with appropriate care. Guidelines for application of synthetic pesticides are provided in SPC documents (e.g. Crop Protection Manual for Trainees, Honiara, Solomon Islands 2012).

3.5 Biological control

Biological control is the use of natural enemies (predators, pathogens, 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), including pathogenic viruses and fungi, predatory carabid and elaterid beetles and parasitic *Scolia* wasps. The relative impact of each natural enemy species within this native community is poorly known, with additional control strategies often needed to complement biological control in coconut and oil palm plantations. (See IPM section 3.6.)

When CRB-S invaded the Pacific, it 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-S populations (e.g. Hoyt 1963). This process is known as classical biological control (Van Driesche and Bellows 1996). Among many natural enemies introduced to the Pacific, very few predators or parasites established (Caltagirone 1981). Incidental predation by pigs and chickens on CRB larvae may assist with control of this pest and can be useful for control of larvae in household or community waste piles. Local species of generalist arthropod predators (centipedes, beetles, ants) may feed on CRB larvae; however, there is little evidence that this contributes significantly to CRB control (Hinckley 1967). Only one pathogen provided significant control of CRB-S: the *Oryctes rhinoceros* nudivirus (OrNV) discovered in Malaysia by Alois M. Huger. (Huger 2005 summarises the history of virus discovery and its use against CRB.) This virus infects CRB larvae and adults, causing death after 6–30 days. Infected adults are weakened prior to death so that they stop feeding, and their mobility and breeding is reduced. Once established in countries invaded by CRB-S, the virus had a significant impact on CRB populations and reduced palm damage (Huger 2005). Another approach to biological control for CRB was to create a biopesticide from a known pathogen. CRB adults and larvae can be infected by strains of the fungus *Metarhizium majus* (formerly *M. anisopliae* var. *majus*). This fungus has been developed into a biopesticide that can be applied to CRB breeding sites in both the native and invaded range (Bedford 2013).

The recent invasion of CRB-G has changed CRB management wherever it is found. CRB-G is not susceptible to the strain of OrNV introduced originally to control CRB-S in the Pacific (Marshall et al. 2017). A new biological control effort is underway in order to identify strains of OrNV from CRB's native range that are effective against CRB-G. Until an effective OrNV strain is discovered, biopesticides containing *M. majus* are the only option for biological control of CRB-G.

3.6 Integrated pest management (IPM)

Integrated pest management (IPM), is a broad-based approach that integrates practices for economic 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".

SPC has recommended IPM for management of CRB and includes combination of the factors described above.

For coconut palms planted for subsistence, ornamental purposes, or in commercial plantations, IPM for CRB involves: i) monitoring of palm damage (section 2.5) to detect localised CRB outbreaks and check that control is successful; ii) biological control (section 3.5) with OrNV (in the invaded and native range) and other co-evolved natural enemies (in the native range only); and iii) sanitation (section 3.1) to remove organic waste, dead palms and other potential breeding sites. 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 uncontrolled (e.g. after cyclones and tropical storms, when palms are often toppled by high winds and large amounts of green waste is created). Historically, outbreaks of CRB often follow cyclone damage (Jackson and Marshall 2017). A high number of 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. Some additional options may be incorporated into IPM programmes for coconut, particularly for commercial plantations or ornamental palms. These more costly options include pheromone traps to monitor adult beetle activity and complement it with visual surveys of palm damage. 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). Commercial products containing the fungus *M. majus* may be applied to CRB breeding sites that cannot be removed. Insecticide treatments are not recommended for established coconut palms (section 3.4) as there is a potential risk of translocation of insecticides (move within the palm), leaving harmful residues in the coconut. If a recent invasion of CRB is targeted for eradication, insecticides may be necessary for success (Figure 3.6). 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.

For higher value crops, particularly oil palm, the same components are needed as for coconut: monitoring; biological control; and sanitation. 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 (section 3.2) and palm damage (section 2.5), particularly for young palms; ii) biological control (section 3.5) with OrNV (invaded and native range) and other natural enemies (native range) plus application of *M. majus* biopesticides to breeding sites that cannot be removed; and iii) sanitation to remove organic waste (section 3.1), particularly during plantation renewal when large amounts of waste is generated; iv) insecticide treatments (section 3.4) 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. In CRB's native range, an additional strategy is to break up and spread the waste as a thin layer, then plant a fast-growing cover crop, often a legume, over the waste matter (Wood 1968).

A decision tree to identify IPM options for coconut and oil palms is presented below (Figure 3.5). This includes decision points to consider potential for eradication of recent CRB invasions as well as management of established CRB populations.

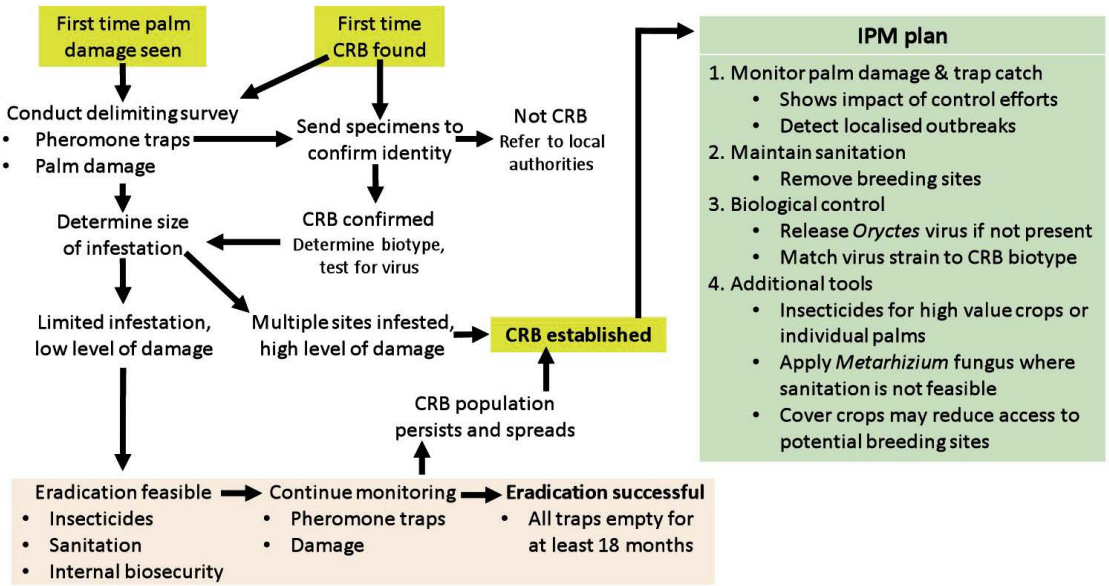

Figure 3.5 IPM decision tree for CRB control in its invasive range. This tree can be used for either CRB-S or CRB-G; however, note that an effective strain of Oryctes virus has not yet been identified for CRB-G.

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Annex 1. Delimiting survey for assessing an outbreak of CRB

Example: Delimiting survey of CRB palm damage in Solomon Islands

Prepared 2019/05/15 by AgResearch with assistance from The Pacific Community (SPC) and Ministry of Agriculture and Livestock (Solomon Islands)

Equipment required

- Trip plan
- Camera with GPS capability (tablet, or cell phones with cameras and GPS tagging also acceptable): Ensure date and time are set correctly
- Power banks to keep camera batteries charged: Ensure fully charged
- Maps of the survey area
- (optional) GPS unit + spare batteries
- 3 CRB pheromone traps plus the lures: The bucket trap system is often a good option (especially with addition of a used copra sack to wrap around buckets); however, other traps also could be used
- Sampling tubes to collect live CRB adults (or larvae where no adults are found): A separate tube for each collection point, bringing enough to avoid running out
- Small tubes for individual collection of interesting samples (bring ~50)
- Bush knife, hand axe, crowbars, spades to dig into logs with suspected breeding sites
- Stationery to record notes by hand and to label containers
- Safety equipment as required by Ministry of Agriculture and Livestock (MAL - Solomon Islands) policies
- Awareness materials for distribution
- Trip report

How to conduct a delimiting survey

- A delimiting survey is to be carried out following a report of CRB damage and/or after a CRB insect (adult or larva) has been collected and positively identified as CRB.
- The trip to a reported area should be planned by Biosecurity Solomon Islands (BSI). The length of time on the island will be determined by local conditions. The extension officer will make local arrangements prior to the trip commencing.
- The end goal is to have a report that informs further clean-up and control of CRB.

Day 1

- Upon arrival at the site where the CRB presence was reported, contact village leaders, as prearranged by the local extension officer.
- Describe the site.
    - Record notes of the site (as per the questions outlined in the KoboCollect CRB form), being sure to record the location by landmark or village as well as by GPS.
    - Photograph the palms in the area (with GPS on!). Take pictures of both CRB damaged and non-CRB palms along with general photos of the area (village, plantation, etc). Ideally, it should be feasible to count 50 palms in the photos taken. Ensure the palm crowns can be seen easily so that any damage (e.g. a silhouette to notches) can be observed.
    - Look for potential CRB breeding sites and, when found, collect live adults. If no adults are found, collect live larvae. (Aim to collect 20-30 beetles from this ground zero site.)
    - Instructions for handling, labelling, storing, and transporting collected CRB are provided at the end of this manual. CRB adults and larvae are to be brought back alive so that tissue samples from them can be dissected and preserved by trained staff at the MAL Henderson site.
- Set up three pheromone traps at the reported invasion site (ground zero) and space the pheromone traps at least 100 metres apart. Be sure to record locations using GPS (or take photos with GPS activated). Follow the directions for use of pheromone traps. Refer to Figure 3.2 where bucket trap are used as an example. Collect trapped beetles at least every second day.

Days 2 to 5 (approximate; timeframe dependent on individual trip plan)

- Using roads or tracks, travel along and look for damage and breeding sites. Initially, record what is seen every 1 kilometre. After 3 kilometres, adjust the frequency as appropriate.
    - Take photos along the way.
    - Travel until no further damage is detected. Photograph the site and mark this on the map. Then go down another road and repeat in all directions from the ground zero point.
    - When breeding sites are identified, record the GPS of the site (also taking note of the location by way of a landmark) and collect adults. If no adults are found, collect larvae. Collect up to 10 beetles per collection site (overall no more than 100 beetles per island). CRB from each collection site should be stored separately. (For further information, please see Annexes).
- If needed, use local knowledge to move to a second (3rd, 4th, etc; based on the trip plan) reported invasion point on the island and repeat the delimiting survey procedures.
- Continue the delimiting survey activities until the area(s) of damage has been fully mapped, as far as is feasible based on time available on the island.

Final day(s)

- Have a team meeting and invite appropriate village leaders and farmers to discuss findings from the trip, share maps, reinforce awareness materials, and introduce initial sanitation and control measures.

Upon return to the head office

- Ensure a report has been written by the team and submitted in a timely manner (i.e. within one week of return).
- Provide CRB samples to the MAL team responsible for dissection and preservation of tissues from the CRB samples. The tissue will then be sent to New Zealand for further processing and analysis.
- Pictures should be downloaded and backed up as soon as possible. The GPS tagged photos are to be made available with the report for further analysis by the appropriate MAL staff.
- ROC will discuss report findings from the delimiting survey and will determine the course of action to be implemented for that island.
- BECC will compile the data from all delimiting survey reports. Where required, this information can be used to prioritise activities nationally (CRB eradication, management, etc).

Annex 2. CRB emergency response plan

During the advent of an outbreak of CRB, mobilisation of the Biosecurity Incident Management System (BIMS) framework of actions is paramount.

Response to incidents will necessitate the establishment of an organisational structure, specific to the management of that incident.

This structure will have two functions or commands:

1. Provision of strategic policy and direction (gold); and
2. Designing, planning and implementing operational activities (silver, bronze and blue field levels).

Gold – members of the national management group

1. Heads of relevant government agencies (ministers or secretary) – Biosecurity NPPO/Agriculture – chair
2. Representation from industry bodies (if any) that may be impacted by the biosecurity incident
3. Local (provincial) government representatives – Also linked to existing National Disaster Management Operations (NDMO) team
4. Chief Plant Protection Officer (CPPO)

Silver – member of the consultative committee

1. The Chief Plant Protection Officer / Chief Technical Officer (Chief Plant Protection Officer or equivalent) – chair
2. Representatives from effected industries (if any)
3. Representatives from other government branches (e.g. plant health and extension services, diagnostics, human health)
4. Representatives of local government – Linked to existing NDMO team
5. Bronze level of command's Operation Manager

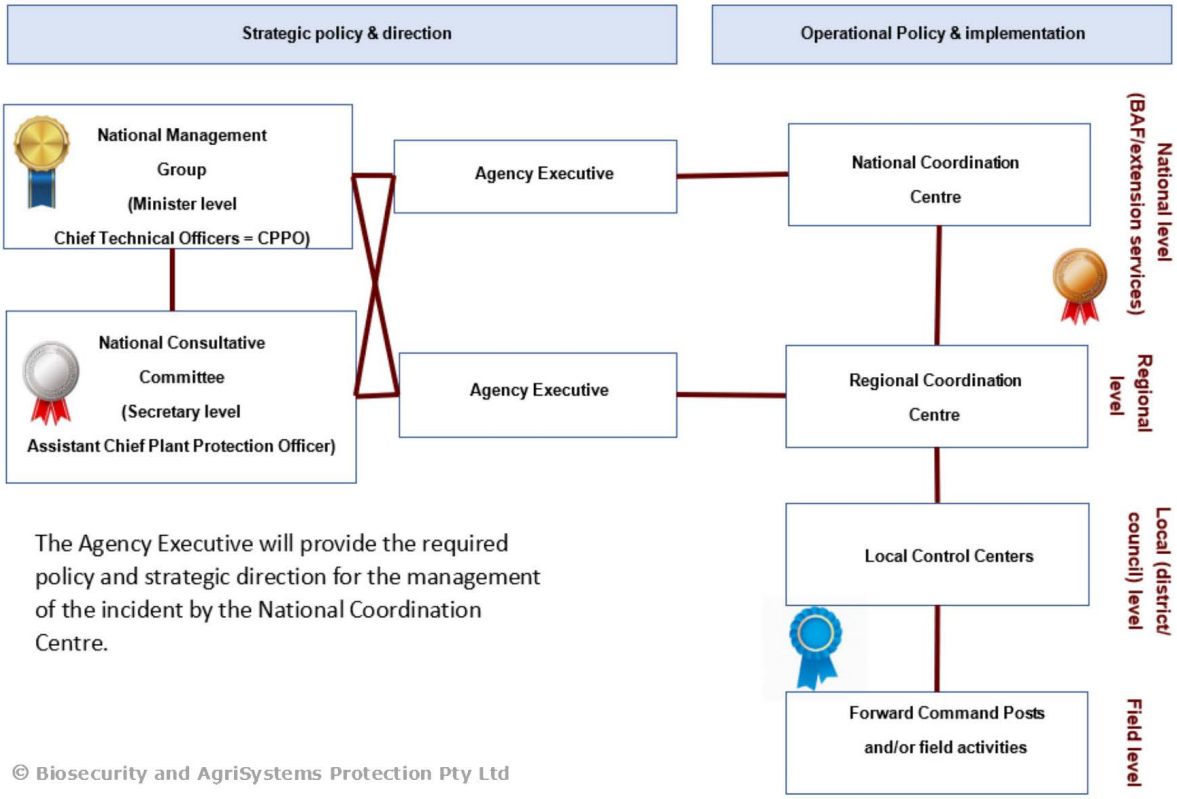
Bronze – member of the local control centre

1. Home of the National Incident Team: ACTION!
2. Head of plant health and/or extension services – chair
3. Operation manager of the National Coordination Centre (if in place)
4. Incident Manager(s) of the National Control Centres
5. Any other relevant specialists (scientists, diagnosticians, etc.)

Operational Policy and implementation

1. Operational policy and implementation are coordinated through the establishment of operations centres at levels appropriate for the particular incident.
2. Coordinated approach to incident response occurs at the following levels:
   i. national level;
   ii. regional or provincial level; and
   iii. local or district (field) level.

Application of Incident Management System



Phases of a response to a biosecurity incident

1. **Investigation and alert phase**
2. **Operational phase**
3. **Stand-down phase**
4. **Relief and recovery phase**

Response 1. Investigation and alert phase

- The investigation and alert phase begins when a notifying party declares that, based on an initial analysis of the pest (or disease), an outbreak (potentially of an Economic Plant Pest (EPP)) exists or **has the potential to exist.**

- During the Investigation and alert phase:
    1. a confirmation of diagnosis is made;
    2. the extent of EPP incursion/outbreak is scoped;
    3. a response plan is prepared (out of the pest-specific contingency plan); and
- Gold and Silver levels of command are convened.

Response 2. Operational phase

- The operational phase begins when the presence of the pest is **confirmed** and activities under a response plan (from pest-specific contingency plan) are implemented.
- The aim of the operational phase is to contain and attempt eradication (if feasible).
- During the Operational phase:
    1. local Control Centres are established.
- At the end of the operational phase:
    2. additional surveillance is needed to demonstrate freedom.

Response 3. Stand-down phase

- The stand-down phase begins when:
    1. the Investigation and alert phase fail to confirm the presence of a pest; or
    2. the response strategy has been effective; or
    3. the eradication of a pest is not considered feasible, cost-effective or beneficial; and
    4. the relevant National Management Group (Gold level) formally declares that the pest outbreak is over.
- Finally, at the end of the stand-down phase:
    1. The National Consultative Committee (Silver level), if established for the response, will conclude its activities and stand down.
    2. The National Management Group (Gold level), if established for the response, will conclude its activities and stand down.

Response 4. Recovery phase

Relief and recovery include coordination of support and the provision of information to affected communities in order to mitigate the impacts of the pest and eradication effort.

This is particularly important if facilities and livestock have been destroyed or livelihoods have been affected by the removal and destruction of crops or cropping systems.

Reference

Perrone TS., 2020. Biosecurity Incident Management System Training; Biosecurity Agri-system Protection Propriety Ltd. Biosecurity Authority of Fiji, November 2019.

Annex 3. Guidelines for field collection of live CRB adults and larvae

Field collection of live CRB adults and larvae

- CRB collection should be carried out at several sites during a delimiting survey.
  - Each CRB collection site requires a separate container.
  - CRB adults should be kept 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 millilitres (~10 x 10 x 10 centimetres). These should hold up to 10 individuals.
  - Small air holes must be made 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.
  - 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 respective stage (adults, 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 cook! Take care not to leave them in an enclosed, unattended vehicle where temperatures can become very hot.
- Transport the beetles back to the MAL staff responsible for dissection and preservation of the tissue samples. These will be sent to the New Zealand team for further analysis.
- Note that, while dissection and preservation of tissue in the field is theoretically possible, you need to have had: 1) the right training to ensure cross contamination between samples is avoided; and 2) 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 samples of interest or an interesting site, 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 useful as fresh specimens.
- Record what you noticed that is unusual compared to healthy beetles you have observed (e.g. infected with fungus (fuzzy coating), odd colouration (e.g. 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, fungal-infected insects (fuzzy coating) do not always indicate death by an insecticidal fungus. Following death, saprophytic 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 someone can revisit the site if it is deemed important.

Annex 4. Collection, preparation and shipment of CRB samples for DNA analysis

Sample collection

- Give each Coconut Rhinoceros Beetle (CRB) specimen a unique reference number.
- Record the 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 millilitre) leak-proof tube.
- Label the tube with the reference number.
- Add 1 millilitre monopropyleneglycol (MPG) to the tube and close the lid.
- Store tubes in the freezer until they are ready to be sent 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 lined with paper towels and seal it.
- Wrap the container in bubble wrap or newspaper and place it into 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 its contents.
- Seal the box and attach labels or place the box in a courier bag and attach labels to the bag.
- Attach labels: 1) a short description of the 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.

Sample shipping label

Contact Details
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 mono-monopropylene glycol (MPG) as a preservative. MPG is not classified as hazardous according to Schedules 1 to 6 of the Dangerous Goods Regulations. [add country-specific regulations here]

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