An Update on the UOG Coconut Rhinoceros Beetle Project

Aubrey Moore
University of Guam
College of Natural and Applied Sciences

April 14, 2021

Contents

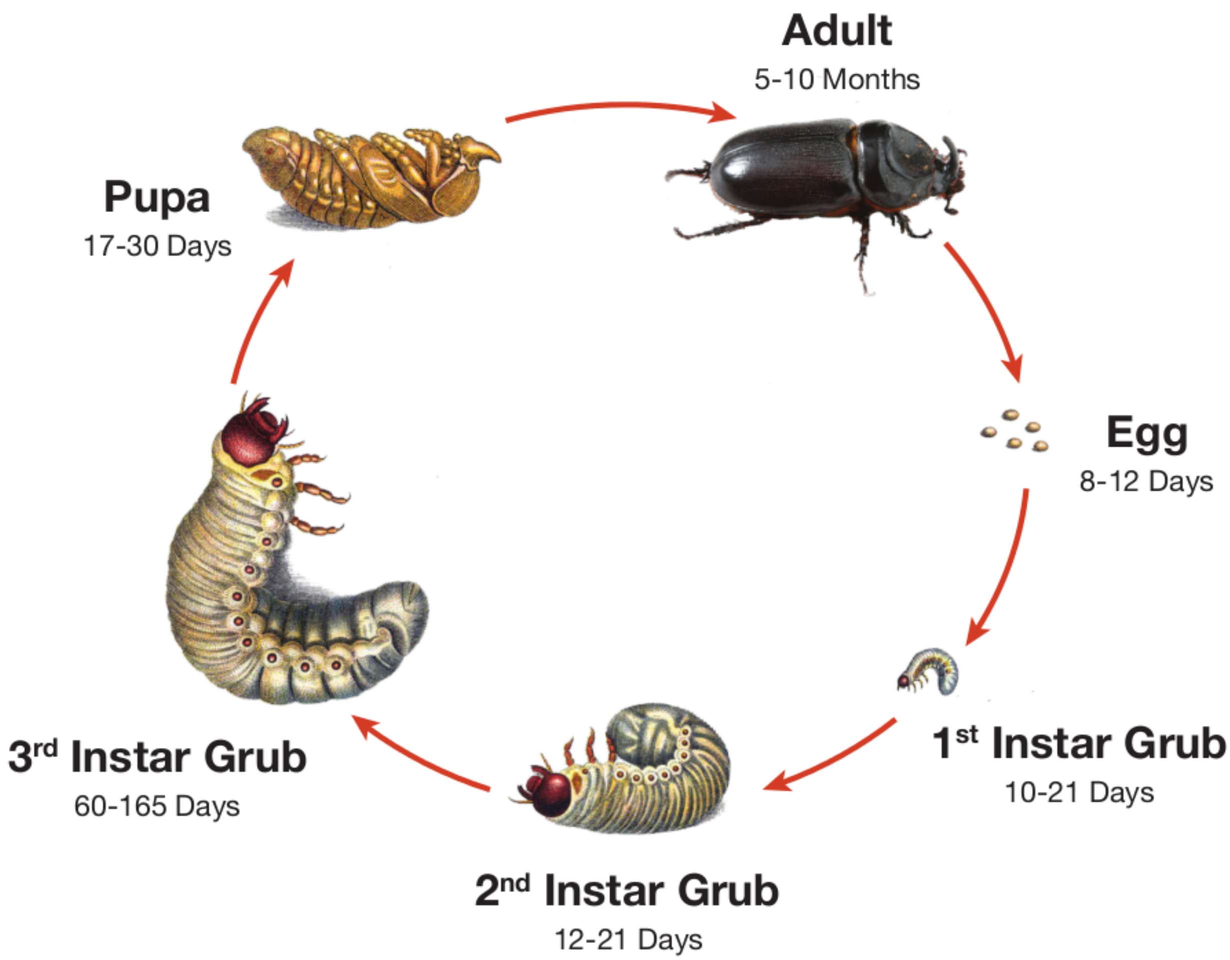


Figure 1: Coconut rhinoceros beetle life cycle.

1 Background

1.1 CRB Biology and Invasion History

CRB is a large scarab beetle native to southeast Asia. Like all beetles, CRB has four life stages: egg, grub, pupa, and adult (Fig. 1). Only the adult stage causes damage. Grubs feed only on decaying vegetation and do no harm. Adult males and females bore into the crowns of coconut palms and other palms to feed on sap. They do not feed on leaves, but they bore holes through developing leaves on their way to the white tissue at the interior of the crown. When these damaged leaves eventually emerge from the crown, they have v-shaped cuts in them, a distinctive sign of CRB damage (Fig. 2). Each adult feeds on sap for only a few days. It then leaves the crown to search for a breeding site. Palms may be killed if a CRB bores through the growing tip (the meristem). Mature palms are rarely killed at low CRB population levels. However, trees are killed when they are simultaneously attacked by many adults during a population outbreak such as the one we are currently experiencing on Guam.

CRB breeding sites can be found in any mass of decaying vegetation. Preferred sites are standing dead coconut stems and fallen coconut logs and fronds. But piles of anything with a high concentration of decaying vegetation can be used as a breeding site including green-waste, dead trees of any species, saw dust, and manure. CRB breeding sites have even been found in commercially bagged soil purchased from a local hardware store [1]. An active breeding site will contain all CRB life stages. Adults locate breeding sites by sniffing out a chemical signal referred to as an aggregation pheromone. This pheromone has been synthesized and is commercially available [2].

A female rhino beetle lays about 100 eggs during her lifetime. Assuming a 50% sex ratio and 100% survival, there will be a population increase of 5,000% during each generation. Thus population explosions may occur when abundant potential breeding sites are available in the form of rotting vegetation following destruction in the wake of a typhoon, large scale land clearing, or war. Large numbers of CRB adults generated by a population explosion may result in large numbers of palms being killed. The dead standing trunks soon become ideal breeding sites which generate even higher numbers of adults. This positive feedback cycle will end when the rhino beetles run out of food, meaning when most of the palms have been killed and rotted away.

CRB invaded islands in the Pacific and Indian oceans during two waves of movement. The first wave occurred started in 1909 when CRB was accidentally transported to from Sri Lanka to Samoa with shipment of rubber tree seedlings and it ended during the 1970s [3]. All of the CRB range expansion during this period was south of the equator except for the invasion of the Ryuku Islands (Japan) starting in 1921 [4] and invasion of the Palau Islands in about 1942 [3]. In Palau, there was a population explosion of rhino beetles because WWII activities created abundant breeding sites. This resulted in about 50% coconut palm mortality overall, and total loss of coconut palms on some of the smaller islands [5].

The second wave of CRB invasions started in 2007 with discovery of CRB on Guam, followed by invasion of Oahu (Hawaii), Port Morseby (Papua New Guinea), Guadalcanal, Savo and Malaita (Solomon Islands), and Rota (Commonwealth of the Northern Mariana Islands). Beetles in the second wave of invasions are genetically different from those in the first wave [6] and these are being referred to as the **Guam biotype** or **CRB-G** for short.

Figure 2: Coconut palms dying after a severe attack from coconut rhinoceros beetles adults.

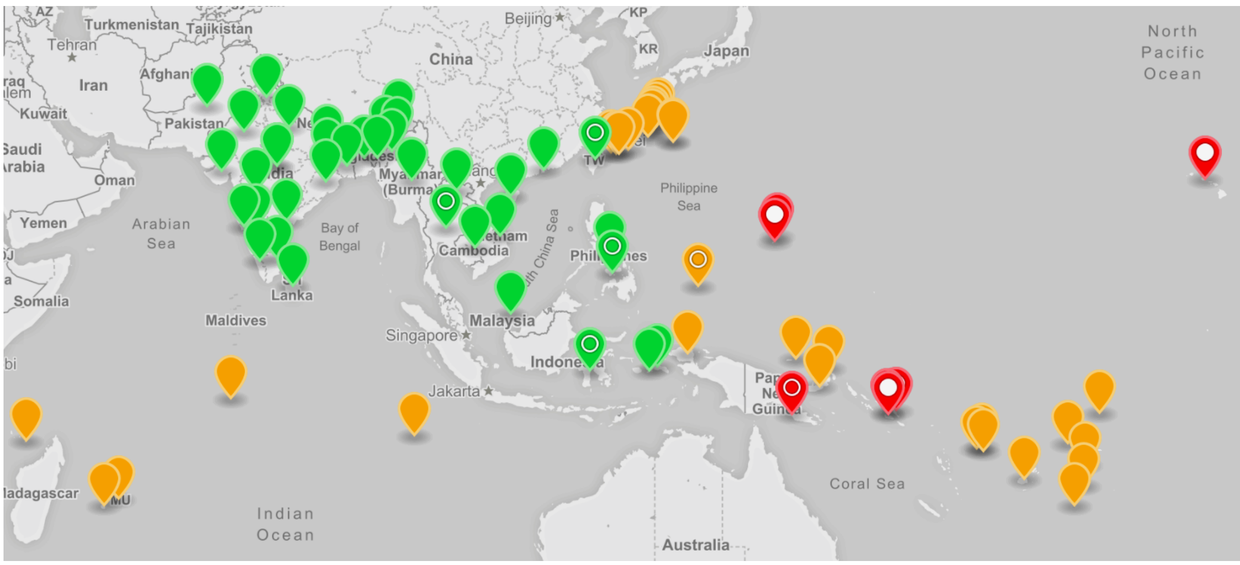


Figure 3: Geographic distribution coconut rhinoceros beetle, *Oryctes rhinoceros*. Green markers: native range; Brown markers: first detected in the 20th century; Red markers: first detected in the 21st century; Open circle: population includes CRB-G biotype; Filled circle: population is exclusively CRB-G biotype. An interactive version of this map is available online at http://aubreymoore.github.io/crbdist/mymap.html.

1.2 Guam CRB Eradication Program

CRB was first detected on Guam was found in Lower Tumon on September 11, 2007. An island-wide survey, completed within two weeks, located CRB grubs and adults and damage symptoms only in Lower Tumon and at the adjacent Faifai Beach, an area totaling less than 1,000 acres [7]. Based on this information it was decided that eradication would be attempted. Here, I am using the word eradication in the proper sense, meaning killing every single CRB on the island.

In theory, a CRB population can be eradication from an island by locating and destroying all active breeding sites and ensuring that the arrival pathway is blocked to prevent re-infestation. In practice, CRB eradication is difficult. There have been several CRB eradication attempts, but only one of these was successful. CRB was eradicated from Niuatoputapu Island, also known as Keppel Island, a tiny outer island of Tonga, only 16 square kilometers in area. Eradication was accomplished by a sanitation program which lasted 9 years following first detection of CRB in 1921 [3].

The Guam CRB Eradication Project was a joint effort involving the United States Department of Agriculture, the Guam Department of Agriculture and the University of Guam. Financial support came from the United States Department of Agriculture, the United States Forest Service and the Legislature of Guam. The project used several tactics aimed at wiping out the CRB population: quarantine, sanitation, trapping, and chemical control. These are explained below.

The opportunity to eradicate CRB from Guam was lost when the infestation spread from the Tumon Bay area to breeding sites on other parts of the island prior to 2010. Most breeding sites are currently inaccessible for application of eradication tactics, being in the deep jungle and/or on military property which includes about one third of the island.

1.2.1 Quarantine

The Guam Department of Agriculture drew a line around the CRB infestation area and required inspection and/or treatment of any dead vegetation being transport to other parts of the island. The quarantine had to be expanded several times. By 2010, all parts of Guam were infested by CRB.

1.2.2 Sanitation

Sanitation is the most important tactic in any CRB eradication project. The target is to find and destroy all breeding sites before adults are generated, thus halting reproduction preventing all damage. The eradication program employed 4 detector dogs trained to sniff out rhino beetle grubs.

1.2.3 Trapping

At the start of the eradication project, we were advised that the adult population could be annihilated using the commercially available aggregation pheromone as a lure. To the contrary, we soon learned that the traps were ineffective for population suppression when new damage appeared in mass trapping areas. However, an island-wide trapping network of about 2,000 traps was useful for monitoring the spread and growth of the CRB population. Island-wide trapping was discontinued when Typhoon Dolphin visited the island in May 2015.

A lot of work was done to improve pheromone traps [9]. Our best pheromone trap design catches about 20 times as many beetles as the original standard trap we started with. However, even these traps catch only about 25% of the adult beetles active in mass trapping areas: not high enough for effective population suppression under current conditions. During our CRB trap improvement project, we discovered that local fishermen were using a small fish gill net called tekken to capture CRB adults emerging from compost piles. This has become a useful tool for managing CRB. Tekken captures about 65% of adults attempting to leave infested compost or green-waste piles. What may be more important is that these same piles become attractive to incoming adults which are also trapped.

1.2.4 Chemical control

Individual palms can be protected from CRB attacks by prophylactic insecticide application. But this is very expensive. A row of 33 severely damaged coconut palms at the University of Guam Agricultural Experiment Station in Yigo were nursed back to apparent perfect health by spraying their crowns with the insecticide cypermethrin on a biweekly schedule. It took 15 months of treatment before all damaged fronds were replaced by healthy ones.

1.3 Guam CRB Biological Control Program

In their native environment insect populations are suppressed by natural enemies which include parasites, predators and pathogens. When alien insects invade islands they escape control by natural enemies, resulting in damaging population explosions. Biological control programs introduce biocontrol agents which specifically target invasive species. There are many examples of successful biological control programs on Guam where invasive species populations are maintained at low levels by purposefully introduced biocontrol agents.

The Guam CRB Biological Control Program was launched following failure of the eradication attempt. There are two widely used biocontrol agents for CRB, a virus called *Oryctes rhinoceros* nudivirus (OrNV) and a fungus called *Metarhizium majus*. Both of these pathogens attack only rhino beetles and pose no risk to other organisms.

1.3.1 Biocontrol Agent 1: *Oryctes rhinoceros* Nudivirus (OrNV)

Shortly after its discovery in Malaysia in 1963 [6], OrNV (*Oryctes rhinoceros* nudivirus), was released as a biocontrol agent on Pacific islands which had been invaded by CRB. The release technique, referred to as auto-dissemination, is very simple: adult rhino beetles are infected with the virus and then released. The virus quickly spreads throughout the CRB population and persists in the environment indefinitely. Wherever the virus was released, there was a drastic decline of the beetle populations followed by a conspicuous recovery of the badly damaged coconut stands. After OrNV was released in Palau, CRB damage symptoms disappeared almost entirely. We expected the same results on Guam.

OrNV was sourced from AgResearch New Zealand, a NZ government research lab which maintains a collection of OrNV isolates. One isolate was imported and released on Guam under conditions of permits from USDA-APHIS and the Guam Department of Agriculture. Unexpectedly, we observed no response to treatment at field release sites. Subsequent laboratory tests showed that the Guam CRB population is resistant to the OrNV isolates available from AgResearch. This discovery lead to the realization that we are dealing with a genetically distinct CRB which is resistant to biological control by OrNV, which has been named CRB-G [6]. Although the **G** refers to Guam, genetic evidence shows that this biotype evolved thousands of years ago, prior to its arrival on Guam.

As previously mentioned, all CRB invasions of Pacific islands in the 21st century involve CRB-G. The current resurgence of CRB damage in Palau is thought to be caused by a recent invasion of the islands by CRB-G rather than by a different biotype which has been there since WWII.

1.3.2 Biocontrol Agent 2: *Metarhizium majus*

Metarhizium majus is a fungus which acts as a pathogen in rhinoceros beetles. Spores of *M. majus* are produced in a laboratory using corn or rice as a substrate and the resulting material is applied to breeding sites as a bio-insecticide.

M. majus was imported from the Philippine Coconut Authority and released on Guam under conditions of permits from USDA-APHIS and the Guam Department of Agriculture. There were no observations of CRB grubs or adults infected with *M. majus* prior to release of the fungus. An extensive post-release survey showed that between 10% and 38% of field collected CRB died from M. majus infection within 21 days after collection. *M. majus* has established on Guam and it is often found in untreated breeding sites. However, this biocontrol agent did not suppressed populations enough to prevent the current outbreak.

1.4 Typhoon Dolphin Triggers a CRB Population Explosion

When we thought that the Guam CRB problem could not get worse, it did. Typhoon Dolphin triggered the current CRB outbreak we are now experiencing.

Typhoon Dolphin visited Guam in May 2015. It was not a very strong typhoon by Guam standards, but it was the first one in more than a decade and it created a lot more damage than expected. Abundant piles of decaying vegetation became CRB breeding sites. Some of these new breeding sites were in villages were they could be managed. But most were inaccessible: in jungles and/or on military land. Within a few months, massive numbers of adults were emerging from breeding sites and severely attacking palms which started to die. Prior to Dolphin, we saw some heavily damages palms, but very few dead ones. Once a palm is killed, its dead standing trunk becomes an excellent breeding site which eventually produces even more adults resulting in a self-sustaining outbreak such as the one we are experiencing.

1.5 Where do we go from here?

If we do not control the current rhino beetle outbreak on Guam, it will only end when the beetles run out of food. Which means most of Guam's palm trees will be killed, as happened in Palau after WWII. If current CRB-G outbreaks in the Pacific cannot be suppressed, it is only a matter of time until this biotype invades other islands through accidental transport. If CRB-G reaches atolls where the coconut palm is the tree of life this will be a human tragedy, possibly displacing islanders to larger population centers.

At the 2016 International Congress of Entomology, the USDA sponsored a meeting to plan a regional response to CRB-G [1]. Pacific-based entomologists with extensive experience working with CRB agreed that our best bet for stopping CRB-G outbreaks is to find an effective biocontrol agent for CRB-G. Most likely this will be an isolate of OrNV which is highly pathogenic for CRB-G. Although a regional project has yet to be funded and organized, the search has already begun. The University of Guam has been awarded a grant from USDA-APHIS to collaborate with rhino beetle biocontrol experts at AgResearch New Zealand and another grant from Department of the Interior's Office of Island Affairs will fund a post-doc entomologist to work on this project.

[8]

2 Current Funding

Since CRB was first detected on Guam in 2007, UOG-CNAS has continually collaborated with Guam DoAg and local USDA-APHIS staff with grant funding from various sources including:

- USDA Animal and Plant Inspection Service (USDA-APHIS)
- US Forest Service (USFS)
- Department of the Interior - Office of Insular Affairs (DOI-OIA)
- Guam Legislature
- Western Integrated Pest Management Center

Until recently, most financial support for UOG CRB project came from USDA-APHIS Plant Protection Act funds (previously Farm Bill funds). However, despite the fact that CRB damage on Guam is much more severe than that on Oahu, APHIS financial support for CRB work on Guam and Hawaii has become extremely disproportionate. During FY2020-21, Guam Department of Agriculture was granted $140,000 from PPA funds for CRB work and two UOG proposals were rejected. During this same period, Hawaii Department of Agriculture received $5,342,066 PPA funding for CRB work.

fix end dates

Funding Source	Amount	Title	Start date	End date
USDA-APHIS	$200.000	Biological control of Coconut Rhinoceros Beetle Biotype G on Guam	2019-08-08	2021-08-07
DOI-OIA	$239,994	Establishment of Self-sustaining Biological Control for Coconut Rinoceros Beetle Biotype G in Micronesia	2020-05-14	2023-09-03
USFS	$98,240	Establishment of Self-sustaining Biological Control of Coconut Rhinoceros Beetle Biotype G in Micronesia	2020-06-17	2021-05-30
USFS	$23,000	Improving Coconut Rhinoceros Beetle Breeding Site Detection Using Harmonic Radar	2020-06-17	2021-05-30

3 Current Staffing

PI: Dr. Aubrey Moore, Entomologist

PostDoc: Dr. James Grasela, Insect Pathologist

Technician: Christian Cayanan

4 Recent Progress

The following subsections were extracted form the latest USDA-APHIS progress report for the project [9]. Note that this report and almost all documents generated by the project are available online.

4.1 Objective 1: CRB Control

The primary objective is to find an *Oryctes rhinoceros* nudivirus (OrNV) isolate which can be used as a highly effective biological control agent for long-term suppression of CRB-G populations. As soon as laboratory studies indicate discovery of an OrNV isolate which is a potential biological control agent for CRB, we will multiply the virus *in vivo* and initiate field releases under the conditions of an existing USDA-APHIS permit.

We have attained little progress towards completing our primary objective, which is to find an isolate of OrNV which is a promising candidate for biocontrol of CRB-G, because of COVID-19 travel restrictions and technical problems with our bioassays. Impediments towards progress are discussed in detail in Section 5.

4.1.1 Regional Collaboration

Work will continue with colleagues at AgResearch New Zealand, the Secretariat of the Pacific Community (SPC), Tokyo University, the University of Hawaii and others to put together a regional collaboration with the objective of finding an effective biocontrol agent for CRB-G.

Project staff promoted Pacific-wide collaboration among people trying to find solutions to the CRB problem by helping to organize professional meetings and by providing online tools to facilitate sharing of scientific and technical information.

CRB Action Group Meeting; December 9, 2020 This meeting was run as a Zoom webinar hosted by the University of Guam. A recording is available online.[10]

Agenda and Presentations:

- Regional Reports
- **Aubrey Moore**: Automated roadside video surveys for detecting and monitoring CRB damage to coconut palms. [11]
- **Sarah Mansfield**: Bioassays with OrNV: Progress and pitfalls.
- **Madoka Nakai and Shunsuke Tanaka**: Characterization of a Palauan isolate of *Oryctes rhinoceros* nudivirus (OrNV).
- **Kayvan Etebari**: Transcriptomic responses of different geographical populations of coconut rhinoceros beetles to *Oryctes rhinoceros* nudivirus (OrNV) infection.
- Open Discussion

CRB Action Group Meeting; March 17, 2021 This meeting was run as a Zoom webinar hosted by the University of Guam. A recording is available online [12].

Agenda and Presentations:

- Regional Reports
- **Sulav Paudel**: Review paper on biological control of CRB
- **Mark Ero**: Evaluation of CRB-G pheromone from the Solomon Islands
- **Lastus Kuniata**: Insecticide work in young oil palm in Solomon Islands
- **Katayo Sagata**: Evaluation of integrated management options in an oil palm cropping system in PNG
- Open Discussion

Rota CRB Eradication Program The PI made a presentation in a webinar organized by Department of the Interior - Office of Insular Affairs (DOI-OIA) entitled *CRB Biology: Know Your Enemy* [13]. This webinar will be converted into a podcast and published online by DOI-OIA which is supporting the Rota CRB Eradication Project with a $250,000 grant.

LISTSERV The PI established a LISTSERV to facilitate email discussions among members of the CRB Action Group.[14]

Online CRB Reference Library Project staff maintain an online reference library of technical and scientific information about coconut rhinoceros beetle.[15]

4.1.2 Foreign Exploration for an Effective Biocontrol Agent for CRB-G

Foreign exploration in search of a microbial biocontrol agent for CRB-G is already underway. During January, 2017, Moore, Iriarte and Marshall collected an isolate if OrNV from a CRB-G population in Negros Island, Philippines. Laboratory bioassays indicate that this isolate is not a good candidate for biocontrol.

We are currently performing laboratory bioassays to evaluate two novel isolates obtained from AgResearch New Zealand. In addition we are attempting to isolate OrNV from CRB adults collected in Taiwan. This population was targeted because Dr. Shizu Watanabe, University of Hawaii, reported an 82% OrNV infection rate in CRB-G collected from this island.

Our next target population is CRB-G found on the southern islands of Japan. We plan to collaborate with Dr. Madoka Nakai, Tokyo University of Agriculture and Technology, to obtain CRB-G/OrNV specimens from these islands.

The project applied for and was granted USDA-APHIS permits to import *Oryctes rhinoceros* [16] and *Oryctes rhinoceros* nudivirus (OrNV) [17].

No progress was made on foreign exploration for OrNV isolates as potential biocontrol agents for CRB during the period covered by this report because of COVID-19 travel restrictions. See section 5.1 for details.

4.1.3 Establish Lab Colonies of CRB-G and CRB-S

We will establish sustainable laboratory colonies of CRB-G and virus susceptible beetles (CRB-S) as a source of healthy beetles for bioassays.

Note: Establishment of a CRB-S colony is contingent on receiving a USDA-APHIS import permit to import live coconut rhinoceros beetles. I requested a permit on March 19, 2019 (Application number P526-190319-001) to replace a previous permit, P526P-11-01844, which I accidentally allowed to lapse into oblivion after only one shipment.

If we are allowed to import CRB-S, this will allow us to do comparative studies to:

- Measure difference in susceptibility to OrNV isolates. (Resistance of CRB-G to OrNV has not yet actually been proven by comparative bioassays.)
- Test for behavioral differences. (It has been hypothesized that the aggregation pheromone, oryctalure, is less attractive to CRB-G than CRB-S.)

The project applied for and was granted a USDA-APHIS permit to import *Oryctes rhinoceros*, Biotype CRB-S [16], [18].

No progress was made on establishing laboratory colonies for CRB-G and CRB-S biotypes during the period covered by this report because of COVID-19 travel restrictions and University of Guam closures. See section 5.1 and section 5.2 for details.

4.2 Objective 2: Establish a Sustainable Coconut Palm Health Monitoring System

The CRB-G outbreak on Guam is currently unmonitored on an island-wide basis. An island-wide pheromone trapping system, using about 1500 traps, was operated by the University of Guam from 2008 to 2014. This monitoring system was transferred to the Guam Department of Agriculture which abandoned the effort at the end of February, 2016.

Currently, many coconut palms are being killed by CRB-G. But, in the absence of a monitoring system, we do not have an estimate of tree mortality or whether or not the damage is increasing or decreasing. Clearly, establishment of a monitoring system is necessary if we want to evaluate success of the proposed biocontrol project, or any other mitigation efforts.

Rather than re-establish a trapping survey, we intend to establish a monitoring system to track temporal and spatial changes in the extent of CRB damage to Guam's coconut palms. Damage symptoms such as v-shaped cuts to fronds, bore holes, and dead standing coconut palm stems are readily observed during roadside surveys. Survey data will be collected using a digital video camera mounted on a truck. Initially, video images of coconut palm damage by CRB-G will be detected, classified and tagged by a technician. When a large number of images have been tagged, these will be used to train a fully automated CRB damage detection and monitoring system. This automated system may be useful as an early detection device for CRB. Roadside surveys on Guam will be performed bimonthly.

Bimonthly automated roadside video surveys for CRB damage are now operational on Guam and the system has been tested on Rota. Videos recorded with a smart phone attached to a vehicle are analyzed using custom-designed artificial intelligence software which recognizes coconut palms and measures CRB damage. A nontechnical description of the survey method is given in the next section.

A presentation on this new CRB survey methodology was made at the December 9 2020 meeting of the CRB-G Action Group conducted as a Zoom webinar [12], [11].

Guam Roadside Video Survey 1 The following four images were extracted from a draft of the University of Guam's Western Pacific Tropical Research Center impact report for 2020.

They provide a nontechnical overview of the new automated roadside video survey for CRB damage and results from the first Guam survey in October, 2020.

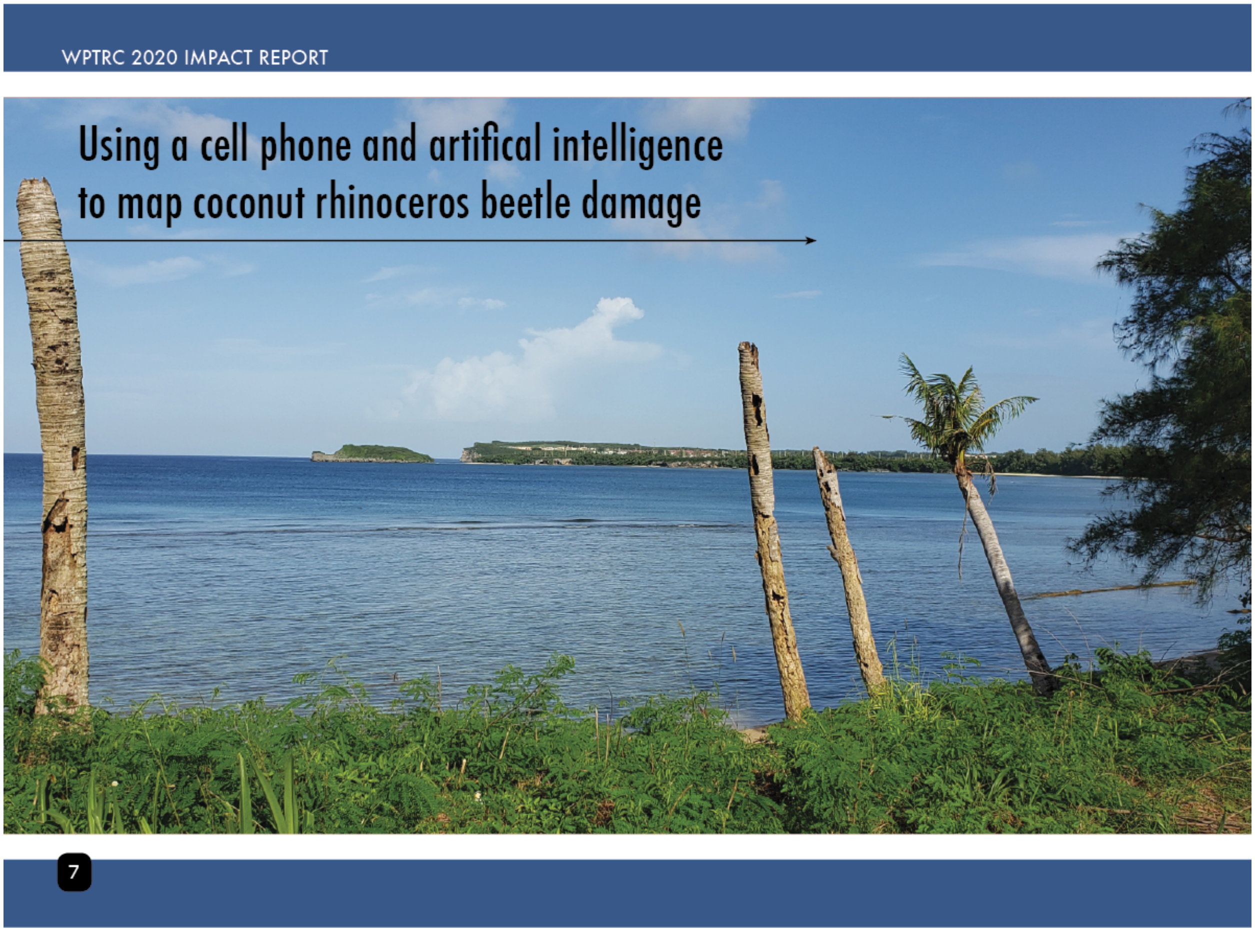


Figure 4: Feature article in the University of Guam's Western Pacific Tropical Research Center impact report for 2020.

Everyone living on Guam has seen damage to coconut palms caused by coconut rhinoceros beetles (CRB). CRB has been on Guam since 2007, however, until recently, the number of palms being damaged and killed on Guam was unknown. Standardized surveys of CRB damage are needed to monitor changes over time and space, especially in response to control activities and for early detection of CRB in new geographic areas.

UOG entomologist Aubrey Moore has developed a highly automated method for routine island-wide monitoring of CRB damage using a cell phone and artificial intelligence (AI).

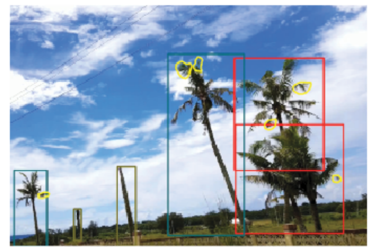
Methods for monitoring CRB damage have been developed. But these rely on direct observation or image analysis by human experts and are too time-consuming and expensive for routine monitoring over large areas.

Dr. Trevor Jackson, an entomologist working for AgResearch New Zealand, has developed a survey method based on a five-level scale for classifying CRB damage to individual coconut palms. Jackson's method is being used extensively on CRB-infested islands in the South Pacific. Moore decided to develop an island-wide roadside CRB damage survey for Guam based on an automated version of Jackson's method.

A smart phone is attached to a vehicle using a magnetic mount. As the car travels, the phone records videos that are analyzed by open-source software.

In the automated survey, a smart phone mounted on a car or truck records continuous videos while the vehicle is driven along all major roads on Guam. The smart phone uses a couple of free apps: OpenCamera records videos and GPSLogger records GPS coordinates.

Recent technical breakthroughs in AI have made it much easier to train computers to recognize objects in digital images. Moore collaborated with OnePanel Inc., an AI tech



Above: medium to severe CRB damage detected in the Royal Palms area of Dededo.

Below: each dot on the map represents a video frame in which one or more coconut palms was detected. The image at the top is a frame extract form a video with approximate at coordinates indicated by the camera icon.

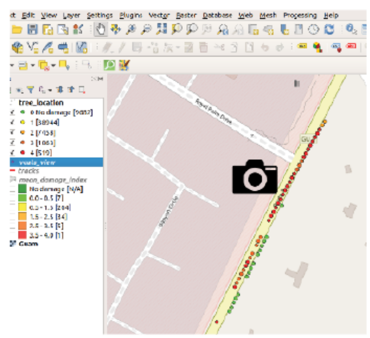


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Figure 5: [Continued] Feature article in the University of Guam's Western Pacific Tropical Research Center impact report for 2020.

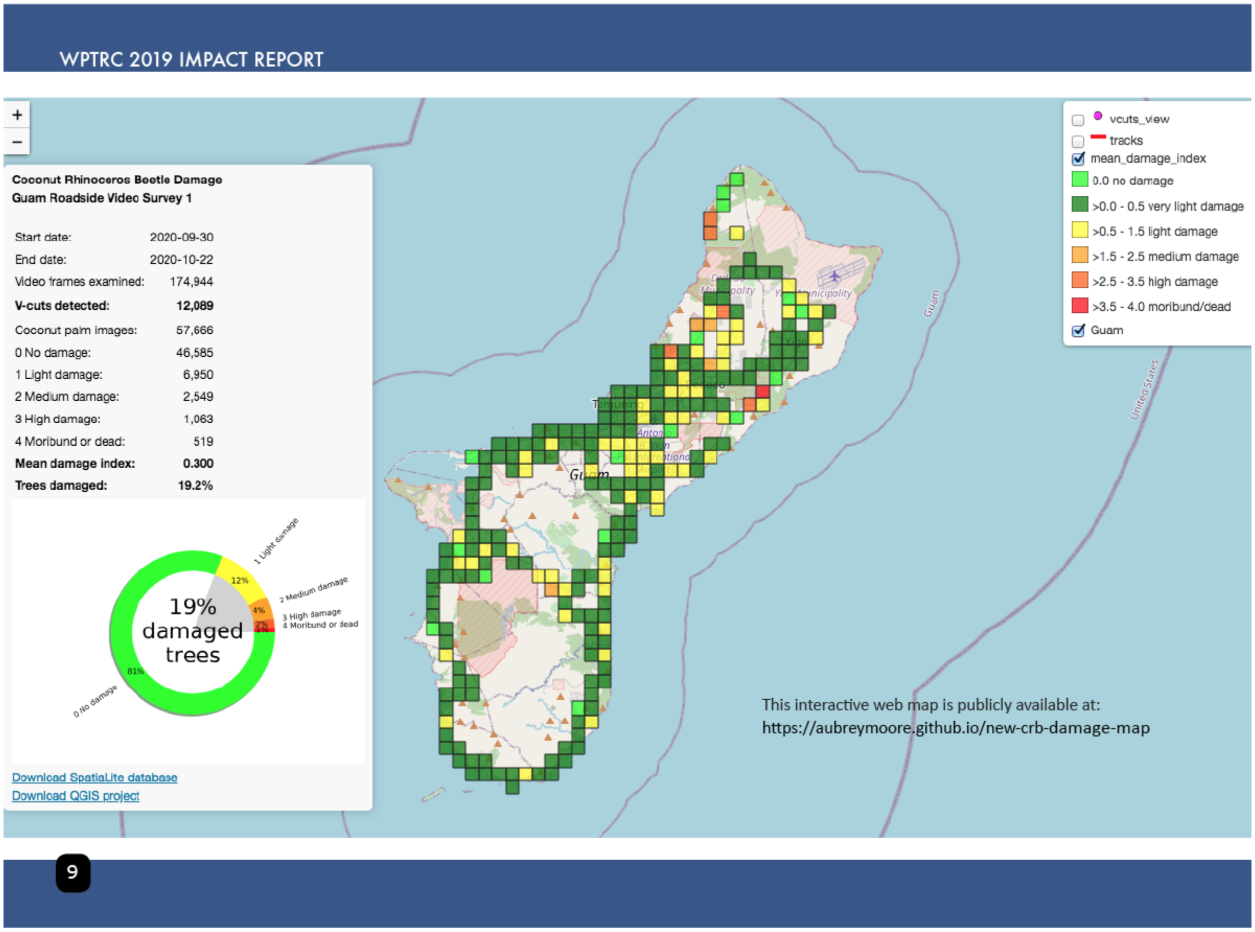

Figure 6: [Continued] Feature article in the University of Guam's Western Pacific Tropical Research Center impact report for 2020. This interactive web map is publicly available at: https://aubreymoore.github.io/new-crb-damage-map

Figure 7: [Continued] Feature article in the University of Guam's Western Pacific Tropical Research Center impact report for 2020.

Guam Roadside Video Survey 2 The proportion of coconut palms damaged by CRB increased significantly from 19.2% in October 2020 to 21.5% in December 2020 ($p < 0.001$; Fisher's exact test).

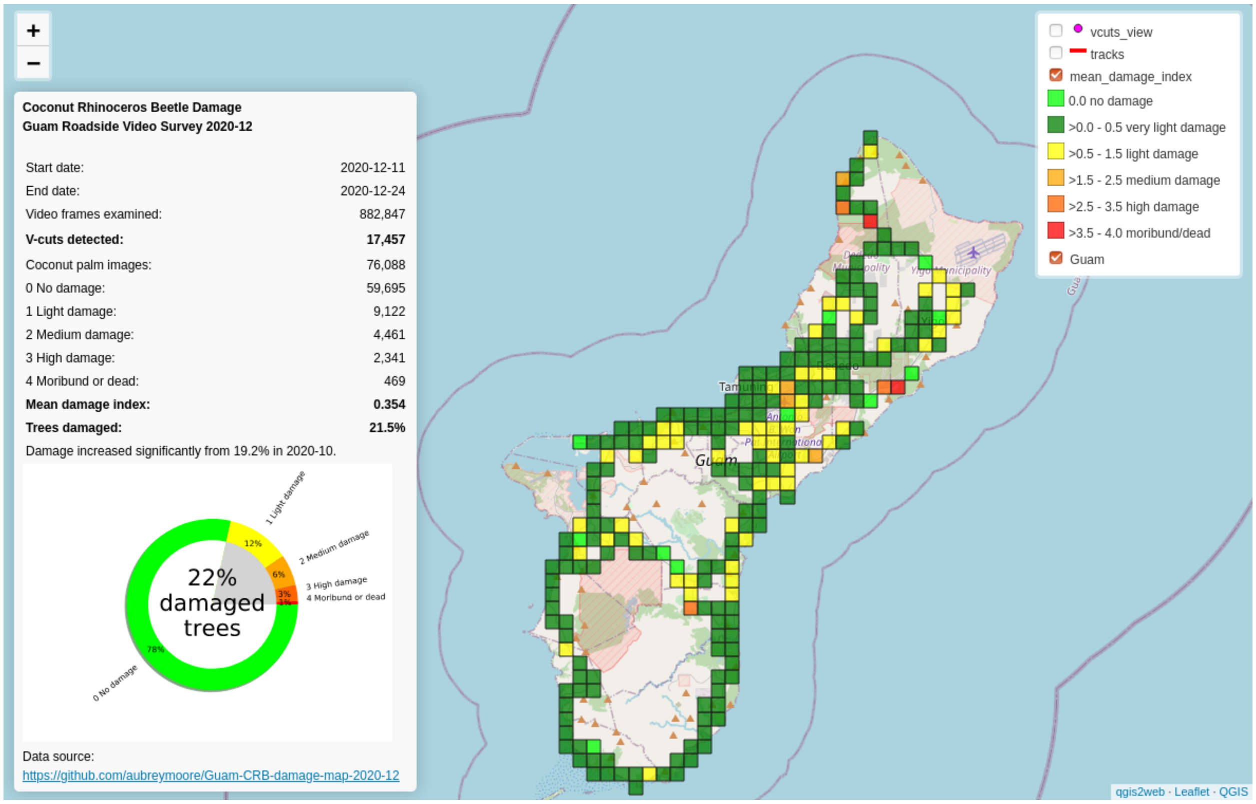

Figure 8: Screenshot of an interactive web map of results from a roadside video survey of CRB damage on Guam in December 2020 https://aubreymoore.github.io/Guam-CRB-damage-map-2020-12/webmap/v1/.

Rota Roadside Video Survey 1 Rota was invaded by CRB in 2017 and eradication efforts by Rota Department of Land and Natural Resources have successfully kept the population at a very low level, although the population has begun to spread to new areas of the island. In October 2020, a smart phone and associated equipment was sent to Rota-DLNR so that they could do an initial roadside video survey in support of their CRB control efforts. In addition to the equipment, a survey setup guide and [19] and a setup video [20] were prepared and sent.

The survey was performed by Mark Manglona, Rota-DLNR and the phone containing videos from the survey was returned to the University of Guam. Videos were analyzed using the workflow developed for the Guam surveys. The resulting web map contained many false positives for CRB damage, but there is one hit which shows a classic v-shaped cut probably caused by CRB. For convenience, data for this hit (images, date, location) were documented as an iNaturalist observation (Figure 9). If this v-shaped cut was caused by CRB, there will be a bore hole. Rota-DLNR located the damaged palm but did not find a bore hole. Therefor the damage was not caused by CRB.

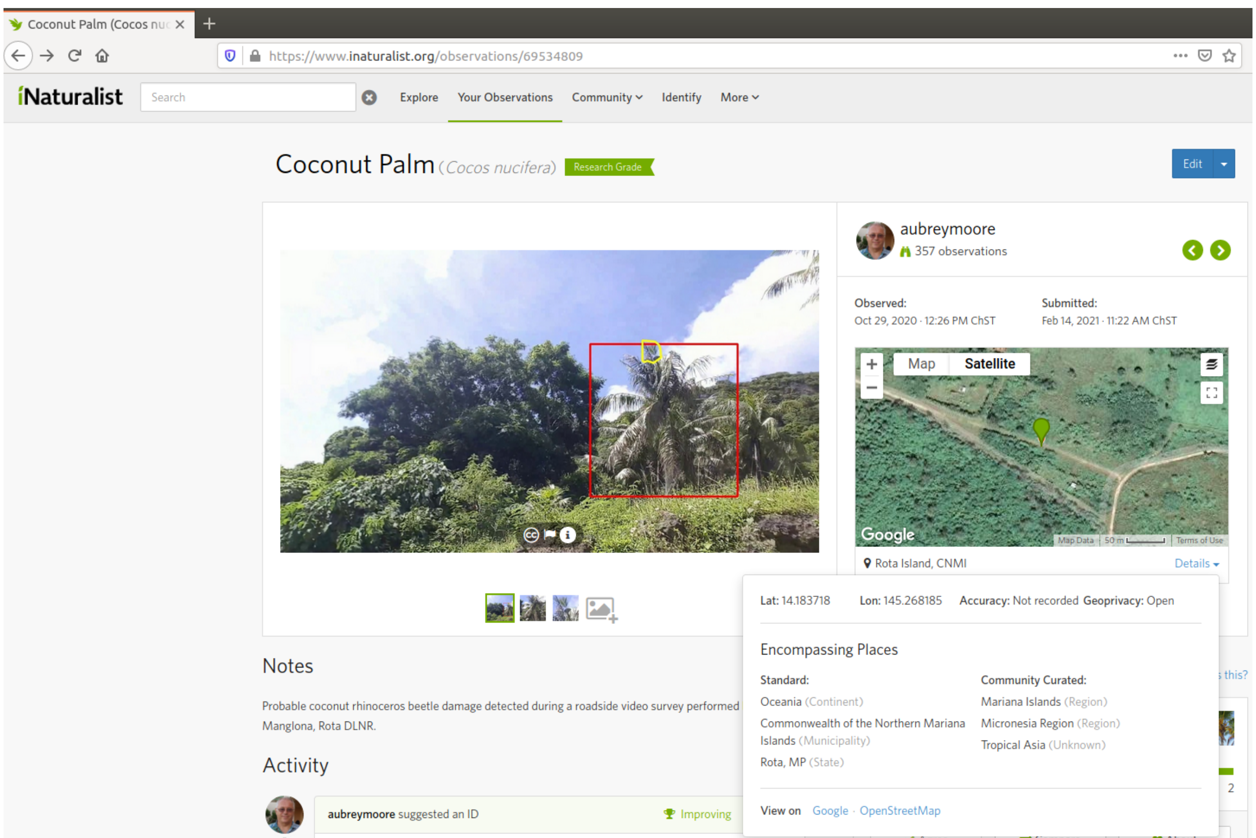

Figure 9: Screenshot of an iNaturalist observation documenting possible coconut rhinoceros beetle damage detected during a roadside video survey performed by Rota DLNR. https://www.inaturalist.org/observations/69534809.

5 Impediments to Progress in the Guam CRB Biocontrol Program

Significant progress was made on the Damage Survey and Regional Collaboration components of this grant. However, progress on the Biological Control component is lagging because of travel restrictions, stay at home orders, and a major technical problem.

5.1 Delays Caused by Travel Restrictions

The original work plan included foreign travel to collect novel OrNV isolates for evaluation as biocontrol agents and to collect virus susceptible CRB biotypes for comparative bioassays. Our first planned collecting trip was to visit American Samoa in December 2019. This trip was canceled at the last minute because of a measles outbreak. Our second attempt, in March 2020 was also canceled at the last minute, because of COVID-19 travel restrictions.

5.2 Delays Caused by COVID-19 Stay-at-Home Orders and University Closures

Progress was further delayed by Government of Guam stay at home orders. The University of Guam was officially closed from March 20 to May 10 2020 and again from August 16 2020 to January 15 2021.

5.3 Delays Caused by Detection of OrNV in CRB Collected on Guam

Our lab currently uses CRB-G adults collected from pheromone traps as test animals in bioassays evaluating OrNV isolates as biocontrol agents under the assumption that the Guam beetle population contains only the CRB-G biotype and is free from OrNV infection. In 2019 we gained the capacity to perform PCR in our lab began testing these assumptions. PCR results indicated that field-collected beetles were all CRB-G, but 18% of these tested positive for OrNV.

Based on these results, the PI decided to suspend bioassays until we had conclusive evidence of OrNV infection in the Guam CRB-G population. An experimental plan [21] was developed and executed. One hundred beetles were collected from each of two trapping sites (Leo Palace Resort in Southern Guam and the UOG Ag. Expt. Stn. in northern Guam). Gut samples were obtained from these beetles and tested using PCR in our lab and also in Sean Marshall's lab at AgResearch New Zealand. In PCR results from both labs all beetles tested positive for CRB-G biotype and negative for OrNV infection [22]. We suspect that previous OrNV positive tests were the result of lab contamination (not false positives). During the hiatus in OrNV bioassays, we re-examined our bioassay methodology and have decided to make major changes before moving forward:

Re-establishment of a CRB rearing program. High variance among results from bioassay replicates and high mortality rates are a serious impediment to progress in finding an OrNV isolate which can be used as an effective biocontrol agent for CRB-G. Beetles collected from pheromone traps are not ideal test insects because they vary in age and many are infected with *Metarhizium majus*. We intend to re-establish the Guam CRB rearing program to supply healthy, standardized test insects. Insects will be reared individually in Mason jars and a detailed record will be maintained for each individual. Larvae will be fed a diet of heat-sterilized CRB breeding site material from dead standing coconut trunks. The CRB-G lab colony will be initiated with surface-sterilized eggs and isofemale lineages will be maintained. Because the life cycle of the coconut rhinoceros beetle is about 9 months, there will be a lag time of about one year before the rearing program is fully operational and bioassays can resume.

Measurement of sublethal impacts of OrNV infection. The literature indicates that reduction in damage to coconut palms after release of OrNV may be the result of sublethal impacts on the population rather than mortality. These impacts may include reduction in fecundity, feeding, and flight. Bioassays which measure only mortality may reject promising biocontrol candidates. We already indirectly track feeding by weighing beetles during bioassays but will also include egg counts in future observations so that we can measure changes in fecundity. We are also considering using flight mills to measure flight capability.

Acquisition of virus susceptible biotypes. One or more lab colonies of virus-susceptible biotypes are required for comparative bioassays. We originally planned to source non-CRB-G beetles during foreign exploration for OrNV isolates, but this has not happened because of COVID-19 travel restrictions. Our new plan is to import gravid females from places such as Palau, where one or more virus-susceptible biotypes are known to exist, in addition to CRB-G. Eggs from these females will be used to establish isofemale lineages and these lines will be genotyped using DNA extracted from the imported females.

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