

Coconut Rhinoceros Beetle Biological Control

Aubrey Moore, University of Guam

October 27, 2020

Report ID: AP18PPQFO000C402-PE-SA1-20

Report Type: Final Report

Reporting Period: SA1-20

Performance Period: October 10, 2019 through March 10, 2020

<https://github.com/aubreymoore/FY19-PPA-Report-1/raw/master/PPA18-report-final.pdf>

Abstract

CRB Biological Control. The primary objective of this project is to find an isolate of *Oryctes rhinoceros* nudivirus (OrNV) which can be used as an effective biological control agent for the CRB-G biotype of coconut rhinoceros beetle (CRB).

Laboratory tests indicate that OrNV from two sources can be considered as potential biocontrol agents CRB-G: OrNV isolate V23B maintained in insect tissue culture by AgResearch New Zealand and OrNV in bodies of CRB collected in Taiwan for the current project. Further laboratory testing of these virus samples is underway.

PCR tests of recently collected CRB-G adults on Guam indicate presence of OrNV in this population. This virus could be from OrNV autodissemination early in the current project or from fortuitous introduction.

CRB Damage Survey. A secondary objective of this project is to develop a CRB damage monitoring system.

A digital image analysis system has been developed to detect and quantify V-shaped cuts to fronds and coconut palm mortality caused by CRB. The heart of this system is an object detector, trained by deep learning technology, which locates CRB damage symptoms on frames from georeferenced roadside video surveys. This object detector can be used to automate detection, quantification and to map changes in CRB damage over time and space and can also be used for early detection of CRB invasion.

A working prototype of the system has been built.

Regional Collaboration. Uncontrolled outbreaks of CRB-G is a major problem for Pacific islands. Outbreaks of this highly invasive biotype are damaging and killing palms in Guam, Rota, Hawaii, Palau, Papua New Guinea, and the Solomon Islands. Without effective control of these outbreaks, the problem will spread to other Pacific islands, resulting in a human tragedy when it reaches atolls where islanders still rely on coconut palm as the *tree of life*.

Project resources, time and effort were used to facilitate communication among an *ad hoc* collaboration of entomologists working on the CRB-G problem throughout the Pacific. Project staff participated in a symposium and planning meeting of the CRB-G Action Group at the XIX International Plant Protection Congress in November 2019.

Contents

1	Background	4
2	Staffing	4
3	Biological Control	4
3.1	Laboratory Bioassays of OrNV Isolates	4
3.2	Virus Transmission Experiment	5
3.3	PCR Tests for OrNV Detection	6
3.4	Survey to Determine Incidence of OrNV Infection in the Guam CRB Population	6
3.5	CRB Rearing	7
3.5.1	Urgent Need for a Pathogen-free CRB Laboratory Colony	7
3.5.2	Laboratory Information Management System	7
3.5.3	Acquisition of a Virus-Susceptible CRB Biotype for Comparative Bioassays	7
4	CRB Damage Survey	8
5	Regional Collaboration	8
5.1	Participation in Scientific Meetings	9
5.2	Development of Online Resources	9
	References	10

1 Background

The major goal of this project was to find an effective biological control agent for coconut rhinoceros beetle biotype G (CRB-G).

Prior to arrival of CRB-G on Guam during 2007, coconut rhinoceros beetle infestations of Pacific islands were readily dealt with by classical biological control using *Oryctes* nudiviruses (OrNV), a pathogen specific to rhinoceros beetles. Following a lack of response to release of OrNV on Guam, research showed that the Guam CRB population is a genetically distinct virus-resistant biotype which has become known as CRB-G (Sean D. G. Marshall et al. 2017). This biotype is highly invasive and is causing massive damage to coconut and oil palms after recent invasion of Papua New Guinea and the Solomon Islands. CRB-G has also invaded Oahu and Rota. Eradication attempts have been launched on these two islands.

Additional goals for this project are to establish a CRB damage survey to evaluate efficacy of biocontrol and other tactics, and to maintain and facilitate collaboration with other Pacific island entomologists working to find solutions for CRB-G management.

2 Staffing

Staff for this project currently comprises of the PI, a post-doc and a technician.

- Funding from the Department of Interior, Office of Island Affairs was used to hire an insect pathologist for a 2 year term. Dr. James Grasela was recruited and started work at UOG on June 24, 2018.
- Funding from this USDA-APHIS project was used to hire Mr. Chris Cayanan as a technician. Mr. Cayanan was hired during December, 2019, as a replacement for Mr. Ian Iriarte who resigned to accept another job.

3 Biological Control

3.1 Laboratory Bioassays of OrNV Isolates

Four isolates of OrNV were evaluated as candidate biological control agents for CRB-G in a series of laboratory bioassays. Virus sample preparations came from Dr. Sean Marshall's lab at AgResearch New Zealand where they are maintained in insect cell culture.

DUG42 Collected from Dumaguete, Negros Island, Philippines in 2017

MALB Collected from Malaysia, details not available.

PNG Collected from Rabaul, Papua New Guinea in 1988

V23B Collected from southern Luzon, Philippines in 1980

During laboratory bioassays, we dosed CRB-G adults with samples of the OrNV isolates, and observed mortality and changes in mass for one month. Each beetle was kept in isolation and individual records were stored in a laboratory information system developed for this application (Section 3.5.2).

Bioassay results, displayed in Table 1, indicate that one of the isolates, V23B, is pathogenic for CRB-G when doses are applied by placing droplets of virus suspension on mouthparts of adult beetles.

Table 1: *Oryctes rhinoceros* nudivirus (OrNV) bioassay results summary.

OrNV isolate	bioassay	method ¹	beetles	replicates	virus mortality (p) ²	inactivated virus mortality (p) ³
DUG42	DUG42 (Moore and J. J. Grasela 2019a)	injection	30	2	40% (0.65)	40% (0.65)
MALB	MALB (Moore and J. J. Grasela 2019b)	injection	30	2	50% (0.37)	0% (1.00)
	MALBperOS (Moore and J. J. Grasela 2019c)	per os	13	1	-60% (1.00)	20% (1.00)
PNG	PNG (Moore and J. J. Grasela 2019d)	injection	81	4	90% (0.00)	5% (1.00)
	PNGperOS (Moore and J. J. Grasela 2019e)	per os	21	1	0% (1.00)	0% (1.00)
V23B	V23B (Moore and J. J. Grasela 2019f)	injection	66	4	88% (0.00)	0% (1.00)
	V23BperOS (Moore and J. J. Grasela 2019h)	per os	32	2	80% (0.07)	20% (0.69)
	V23-large_bioassay (Moore and J. J. Grasela 2019g)	per os	53	1	42% (0.00)	-
	V23_perOSIN (Moore and J. J. Grasela 2019i)	per os	16	1	60% (0.06)	-

¹ Adult beetles were dosed either by direct injection of virus suspension into the haemocoel or by applying a droplet containing virus to mouthparts.

² Percent mortality in beetles treated with virus, adjusted for untreated control mortality; number in parentheses is the p -value resulting from a Fisher's exact test of significant difference between mortality of treated and untreated beetles.

³ Percent mortality in beetles treated with heat inactivated virus, adjusted for untreated control mortality; number in parentheses is the p -value resulting from a Fisher's exact test of significant difference between mortality of treated and untreated beetles.

In a separate experiment, macerated guts from OrNV infected beetles from field collections of CRB in Taiwan were fed to CRB-G field-collected on Guam. PCR results indicated that the Taiwanese OrNV propagated in the Guam beetles (See Subsection 3.3).

We now have two isolates of OrNV which can be considered as candidate biocontrol agents for further testing: V23B and Taiwan.

3.2 Virus Transmission Experiment

This experiment was performed to determine if OrNV isolate V23B can be transmitted from a dosed CRB adult to an undosed CRB adult. If a virus is not contagious in the lab, it will probably have no potential as a biocontrol agent.

Unfortunately, the experiment failed due to very high mortality in the experimental control group and results are inconclusive. For details, see J. Grasela and Moore 2020. This experiment needs to be repeated using pathogen-free, laboratory-reared adults (See Section 3.5).

3.3 PCR Tests for OrNV Detection

Previously, our laboratory relied on outside collaboration for molecular testing to determine presence of OrNV in CRB tissues. We recently acquired access to equipment and supplies which allow us to use PCR (polymerase chain reaction) techniques for OrNV detection. We tested five different primer pairs for detection of OrNV in reference samples from AgResearch New Zealand and were successful with all five. Identity of DNA fragments was confirmed as OrNV using a commercial DNA sequencing service.

Details of our PCR technique and initial series of tests can be found in J. J. Grasela and Moore [2020b](#), J. J. Grasela and Moore [2020c](#) and J. J. Grasela and Moore [2020d](#).

Results from PCR tests indicate:

- OrNV is present in the Guam adult CRB population: 18% (10 of 47) gut samples dissected from field collected CRB tested positive for OrNV.
- OrNV is present in the adult Taiwanese CRB population: 7% (5 of 67) gut samples dissected from field collected CRB tested positive for OrNV.
- OrNV from Taiwanese adult CRB propagates in Guam adult CRB: 12% (5 of 41) Guam CRB adults dosed with Taiwanese gut preparations tested positive.

3.4 Survey to Determine Incidence of OrNV Infection in the Guam CRB Population

A survey to determine incidence of OrNV infection in the Guam CRB population was performed following positive PCR tests for OrNV infection in 18% (10 of 47) gut samples dissected from locally collected beetles. The experimental protocol developed to minimize false positive tests is detailed in (Moore, J. J. Grasela, and Sean David Goldie Marshall [2020](#)). Preliminary results (J. J. Grasela and Moore [2020a](#)) indicate that previous PCR tests indicating OrNV infection were most likely caused by laboratory contamination.

3.5 CRB Rearing

Experimental beetles were field-collected on Guam as prepupae, pupae or adults from breeding sites or as adults from pheromone traps. Each beetle was given a serial number and was reared individually in a Mason jar stored in one of three environmental chambers set for 30 degrees Celsius, 80% relative humidity and 12 hour photoperiod. Each adult beetle was fed weekly with a slice of banana. Detailed information on the CRB rearing facility can be found in a document prepared in support of a USDA-APHIS permit to allow importation of live CRB for laboratory bioassays (Moore 2019a).

3.5.1 Urgent Need for a Pathogen-free CRB Laboratory Colony

To date, CRB used in bioassays and other laboratory experiments were field collected on Guam, rather than from reared in a laboratory colony. This was done because a very high population density of rhino beetles on Guam made field collection far more efficient in terms of time and resources than laboratory rearing. However, field collected beetles are often infected with pathogens, especially the fungus, *Anisopliae majus*, which was introduced on Guam as a classical biological control agent. Presence of these pathogens in experimental animals resulted in unpredictable and often high mortality rates in experimental control groups, leading to inconclusive results such as experienced with the recent virus transmission experiment (3.2).

Recent discovery of OrNV within the wild Guam rhino beetle population (3.3) now makes it mandatory to establish a sterile CRB-G laboratory colony to supply experimental animals.

3.5.2 Laboratory Information Management System

We developed an online database which we use as a laboratory information management system for maintaining individual records for beetles in our rearing program (Moore 2019b). This system was developed using the [web2py python web framework](#) and it is available on the web at <http://aubreymoore.pythonanywhere.com/rearing3>.

3.5.3 Acquisition of a Virus-Susceptible CRB Biotype for Comparative Bioassays

Since discovery of the CRB-G biotype on Guam (Sean D. G. Marshall et al. 2017), we have been operating under the hypothesis that this biotype is significantly more tolerant (resistant) to pathogenic effects of OrNV isolates previously used as effective biocontrol agents for CRB invading Pacific Islands. It has also been hypothesized that CRB-G has different behavioral characteristics, such as a significantly reduced attraction to oryctalure. However, comparative laboratory bioassays have not been performed to test these hypotheses.

We applied for and have been granted a USDA-APHIS import permit for live CRB which will allow us to establish a laboratory colony of CRB from presumed non-virus-resistant populations (See (Moore 2019a) and (USDA-APHIS 2019)). We have designed custom shipping containers to facilitate secure transport of live CRB (Moore and Quitugua 2017).

We plan to import CRB from American Samoa with assistance from our collaborator, Dr. Mark Schmaedick, American Samoa Community College. Plans for project staff to visit American Samoa in December 2019 failed because of travel restrictions prompted by a measles outbreak and in March 2020 because of travel restrictions prompted by the corona virus pandemic.

4 CRB Damage Survey

The objective of this component of the project was to develop an automated system to evaluate CRB damage by image analysis of roadside video surveys. We completed a *proof of concept* trial in which deep learning algorithms were used to train an object detector which locates and counts dead and CRB-damaged coconut palms in video streams. Visual results are presented in a YouTube post (Moore 2019d) and technical details are available in an Open Science Framework Project (Moore 2019c).

5 Regional Collaboration

An informal collaboration, the *CRB-G Action Group*, has been formed among Pacific-based entomologists working on the CRB-G problem. Participants from Guam, Hawaii, Palau, Papua New Guinea, Solomon Islands, Fiji, Malaysia, Japan and New Zealand have met several times and future meetings are planned (Table 2). This is an *ad hoc* group which has been organized by Dr. Trevor Jackson and Sean Marshall of AgResearch New Zealand. AgResearch is recognized as a global center for expertise on biological control of CRB. AgResearch scientists have worked on CRB in the south Pacific for several decades and they maintain a library of OrNV isolates in cell culture. The New Zealand government has recently committed several million dollars to aid response to CRB-G in the south Pacific islands. Although individual institutions working to find a solution to the CRB-G problem on American-affiliated islands in the northern Pacific have secured funding from multiple, short-term grants, attempts to secure funding to support a sustainable well-coordinated regional project have been unsuccessful.

Table 2: Meetings of the CRB-G Action Group

2015 Pacific Entomology Conference, Honolulu, HI, USA
2016 International Congress of Entomology, Orlando, USA
2017 Japanese Society for Insect Pathology, Tokyo, Japan
2018 Society for Invertebrate Pathology, Gold Coast, Australia
2019 XIX International Plant Protection Congress, Hyderabad, India
2020 (tentative): Pacific Plant Protection Organization, Guam

5.1 Participation in Scientific Meetings

Moore and Grasela participated at the XIX International Plant Protection Congress in a symposium entitled *The challenge of coconut rhinoceros beetle, *Oryctes rhinoceros*, to palm production and prospects for control in a changing world*. Moore made an oral presentation at this meeting (Moore, J. J. Grasela, and Sean D. G. Marshall 2019). They also participated in a CRB-G Action Group meeting with colleagues from throughout the Pacific and Asia.

5.2 Development of Online Resources

Project resources were used to build and maintain the following:

- CRB Bibliography <https://github.com/aubreymoore/CRB-Bibliography>
- Interactive CRB Invasion History Map <https://aubreymoore.github.io/crbdist/mymap.html>
- CRB Wiki Site <https://guaminsects.net/CRBG>
- CRB-G Facebook Site <https://www.facebook.com/groups/crbg07>

References

- Grasela, James J. and Aubrey Moore (2020a). *Investigation to Determine the Presence of OrNV in the Guam CRB Population*.
- Grasela, James J. and Aubrey Moore (2020b). *Technical Report: Polymerase Chain Reaction (PCR) Analysis of the Coconut Rhinoceros Beetle (CRB), Oryctes Rhinoceros. Part 1*. URL: <https://github.com/aubreymoore/FY19-PPA-Report-1/blob/master/pcrdata/PCR-Summary-Part-1.pdf>.
- Grasela, James J. and Aubrey Moore (2020c). *Technical Report: Polymerase Chain Reaction (PCR) Analysis of the Coconut Rhinoceros Beetle (CRB), Oryctes Rhinoceros. Part 2*. URL: <https://github.com/aubreymoore/FY19-PPA-Report-1/blob/master/pcrdata/PCR-Summary-Part-2.pdf>.
- Grasela, James J. and Aubrey Moore (2020d). *Technical Report: Polymerase Chain Reaction (PCR) Analysis of the Coconut Rhinoceros Beetle (CRB), Oryctes Rhinoceros. Part 3*. URL: <https://github.com/aubreymoore/FY19-PPA-Report-1/blob/master/pcrdata/PCR-Summary-Part-3.pdf>.
- Grasela, James and Aubrey Moore (2020). *Guam CRB Biocontrol Project Technical Report: OrNV Transmission Experiment*. URL: <https://github.com/aubreymoore/OrNV-Transmission/blob/master/ornv-transmission.pdf>.
- Marshall, Sean D. G., Aubrey Moore, Maclean Vaqalo, Alasdair Noble, and Trevor A. Jackson (Oct. 1, 2017). "A New Haplotype of the Coconut Rhinoceros Beetle, *Oryctes Rhinoceros*, Has Escaped Biological Control by *Oryctes Rhinoceros* Nudivirus and Is Invading Pacific Islands". In: *Journal of Invertebrate Pathology* 149, pp. 127–134. ISSN: 0022-2011. DOI: [10.1016/j.jip.2017.07.006](https://doi.org/10.1016/j.jip.2017.07.006). URL: <http://www.sciencedirect.com/science/article/pii/S0022201117300289> (visited on 08/26/2017).
- Moore, Aubrey (June 2, 2019a). *Additional Documentation in Support of a Permit Application for Importation of Live Coconut Rhinoceros Beetles to Guam*. URL: <https://github.com/aubreymoore/CRB-Import-Permit/blob/master/CRB-import-permit-request-additional.pdf>.
- Moore, Aubrey (Oct. 13, 2019b). *Coconut Rhinoceros Beetle Rearing Database*. URL: <http://aubreymoore.pythonanywhere.com/rearing>.
- Moore, Aubrey (Oct. 12, 2019c). *Open Science Framework: Digital Image Analysis for Detection of CRB Damage in Videos*. URL: https://osf.io/czr8t/?view_only=cdec7cc47b514b31b33115113193a2d4.
- Moore, Aubrey, director (July 26, 2019d). *Training an Object Detector to Locate Coconut Palms Damaged or Killed by Coconut Rhinoceros Beetle*. URL: <https://www.youtube.com/watch?v=zzSorqcmt9U> (visited on 10/09/2019).
- Moore, Aubrey and James J. Grasela (2019a). *Bioassay Report: DUG42*. University of Guam. URL: <https://github.com/aubreymoore/rearing3/raw/master/bioassay-DUG42.pdf>.

- Moore, Aubrey and James J. Grasela (2019b). *Bioassay Report: MALB*. University of Guam. URL: <https://github.com/aubreymoore/rearing3/raw/master/bioassay-MALB.pdf>.
- Moore, Aubrey and James J. Grasela (2019c). *Bioassay Report: MALBperOS*. University of Guam. URL: <https://github.com/aubreymoore/rearing3/raw/master/bioassay-MALBperOS.pdf>.
- Moore, Aubrey and James J. Grasela (2019d). *Bioassay Report: PNG*. University of Guam. URL: <https://github.com/aubreymoore/rearing3/raw/master/bioassay-PNG.pdf>.
- Moore, Aubrey and James J. Grasela (2019e). *Bioassay Report: PNGperOS*. University of Guam. URL: <https://github.com/aubreymoore/rearing3/raw/master/bioassay-PNGperOS.pdf>.
- Moore, Aubrey and James J. Grasela (2019f). *Bioassay Report: V23B*. University of Guam. URL: <https://github.com/aubreymoore/rearing3/raw/master/bioassay-V23B.pdf>.
- Moore, Aubrey and James J. Grasela (2019g). *Bioassay Report: V23B Large Bioassay*. University of Guam. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-V23B%5C_large%5C_bioassay.pdf.
- Moore, Aubrey and James J. Grasela (2019h). *Bioassay Report: V23BperOS*. University of Guam. URL: <https://github.com/aubreymoore/rearing3/raw/master/bioassay-V23BperOS.pdf>.
- Moore, Aubrey and James J. Grasela (2019i). *Bioassay Report: V23BperOSIN*. University of Guam. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-V23B%5C_perOSIN.pdf.
- Moore, Aubrey, James J. Grasela, and Sean D. G. Marshall (Nov. 11, 2019). *Presentation: Status of a Major Outbreak of Coconut Rhinoceros Beetle, Oryctes Rhinoceros Biotype G, on Guam and Attempts at Establishing Biological Control. XIX International Plant Protection Congress, Hyderabad, India. Nov. 11, 2019*. URL: https://github.com/aubreymoore/IAPPS-2019-Presentation/raw/master/Moore_IAPPS-2019.odp.
- Moore, Aubrey, James J. Grasela, and Sean David Goldie Marshall (2020). *Experimental Plan: Determining Presence of OrNV in the Guam CRB-G Population*.
- Moore, Aubrey and Roland Quitugua (June 1, 2017). *Container for Secure Shipment of Live Coconut Rhinoceros Beetle Adults*. Guam Coconut Rhinoceros Beetle Project Technical Report CRB-2017-05-27. University of Guam Cooperative Extension Service, p. 3. URL: https://github.com/aubreymoore/CRB-Import-Permit/raw/master/CRB_Shipping_Container.pdf.
- USDA-APHIS (July 5, 2019). *CRB Import Permit*. URL: <https://github.com/aubreymoore/CRB-Import-Permit/blob/master/2019-CRB-Import-Permit.pdf>.