USDA APHIS Grant AP17PPQFO000C312
Final Report
Report ID: AP17PPQFO000C312-PE-Final-19
Performance Period: August 1, 2017 - July 31, 2019

Coconut Rhinoceros Beetle Biological Control

Aubrey Moore, University of Guam

November 3, 2019

https://github.com/aubreymoore/FB17-Final-Report/raw/master/report.pdf

In a Nut Shell

- The primary objective of this project was to find an isolate of *Oryctes rhinoceros* nudivirus (OrNV) which can be used as an effective biological control agent for CRB-G biotype of coconut rhinoceros beetle (CRB). Laboratory bioassays of four isolates indicated that one of them, OrNV isolate V23B, is pathogenic for CRB-G and can be considered as a potential biocontrol agent for this pest.

- A secondary objective of this project was to develop a CRB damage monitoring system. A digital image analysis system was 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.

- 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 were islanders still really 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.

Contents

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* nudivirus (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 [1]. 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 only two people: the PI, Dr. Aubrey Moore, and a post-doc, Dr. James Grasela.

- Dr. James Grasela, an insect pathologist, has been hired for a term of 2 years with a grant from Department of Interior, Office of Island Affairs.
- Ian Iriarte, a graduate student working on this project, resigned to accept a permanent job. Search for a replacement is under way.

3 Bioassays to Detect Candidate Biocontrol Agents for CRB-G

3.1 Bioassay Results

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 were 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 islolates, 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 3.3).

Bioassay results are displayed in Table 1. Results 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[1]	injection	30	2	40% (0.65)	40% (0.65)
MALB	MALB[2]	injection	30	2	50% (0.37)	0% (1.00)
	MALBperOS[3]	per os	13	1	-60% (1.00)	20% (1.00)
PNG	PNG[4]	injection	81	4	90% (0.00)	5% (1.00)
	PNGperOS[5]	per os	21	1	0% (1.00)	0% (1.00)
V23B	V23B[6]	injection	66	4	88% (0.00)	0% (1.00)
	V23BperOS[8]	per os	32	2	80% (0.07)	20% (0.69)
	V23-large_bioassay[7]	per os	53	1	42% (0.00)	-
	V23_perOSIN[9]	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.

Bioassay Technical Reports

[1] Aubrey Moore and James J. Grasela. *Bioassay Report: DUG42*. University of Guam, 2019. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-DUG42.pdf.

[2] Aubrey Moore and James J. Grasela. *Bioassay Report: MALB*. University of Guam, 2019. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-MALB.pdf.

[3] Aubrey Moore and James J. Grasela. *Bioassay Report: MALBperOS*. University of Guam, 2019. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-MALBperOS.pdf.

[4] Aubrey Moore and James J. Grasela. *Bioassay Report: PNG*. University of Guam, 2019. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-PNG.pdf.

[5] Aubrey Moore and James J. Grasela. *Bioassay Report: PNGperOS*. University of Guam, 2019. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-PNGperOS.pdf.

[6] Aubrey Moore and James J. Grasela. *Bioassay Report: V23B*. University of Guam, 2019. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-V23B.pdf.

[7] Aubrey Moore and James J. Grasela. *Bioassay Report: V23B Large Bioassay*. University of Guam, 2019. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-V23B%5C_large%5C_bioassay.pdf.

[8] Aubrey Moore and James J. Grasela. *Bioassay Report: V23BperOS*. University of Guam, 2019. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-V23BperOS.pdf.

[9] Aubrey Moore and James J. Grasela. *Bioassay Report: V23BperOSIN*. University of Guam, 2019. URL: https://github.com/aubreymoore/rearing3/raw/master/bioassay-V23B%5C_perOSIN.pdf.

3.2 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 is given a serial number and is 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. Adult beetles are feed weekly with a slice of banana. Beetles used in bioassays were rearing for at least one month before being dosed. Details 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 [2].

3.3 Laboratory Information Management System

We developed an online database which we are using as a laboratory information management system for maintaining individual records for beetles in our rearing program [3]. This system was developed using the web2py python web framework and it is available on the web at `http://aubreymoore.pythonanywhere.com/rearing`.

3.4 Acquisition of an OrNV isolate from Taiwan

During May 2019, Moore made a trip to Taiwan to receive 80 adult coconut rhinoceros beetles collected by Dr. Frank Hsu of the Taiwan Normal University. The Taiwan population is of special interest because specimens in a previous collection were all determined to be CRB-G and 82% of these tested positive for OrNV [12].

This virus isolated from the recent samples has not yet been tested. Beetle specimens and virus samples have been sent to AgResearch New Zealand for DNA analysis.

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

Since discovery of the CRB-G biotype on Guam [1], 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 [2] and [11]).

We plan to import CRB from American Samoa and have already provide our collaborator, Dr. Mark Schmaedick at American Samoa Community College, with secure shipping containers we designed to facilitate secure transport of live CRB [10].

3.6 Laboratory Improvements

Our current lack of molecular technology for detecting or quantifying OrNV is a major impediment to performing and interpreting bioassays. We ordered laboratory equipment and supplies to remove this impediment and now have the ability to detect OrNV.

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 [7] and technical details are available in an Open Science Framework Project [5].

5 Outreach and Presentations

During Spring Term 2018, we made a presentation on the Guam CRB problem [1] and lead field trips for a Guam Humanities Project entitled *Taking Root: Growing Youth Empowerment for Island Sustainability*.

During Fall Term 2018, we did monthly presentations on coconut rhinoceros beetles and other insects for young children participating in the Guam Head Start Program. In addition, a magazine article was published [5] and four oral presntations relevant to the project were made at scientific meetings [2, 4, 3]

[1] Aubrey Moore. "The Coconut Rhinoceros Beetle Outbreak on Guam: What Can Be Done About It?" In: Guam Humanities Project: Taking Root: Growing Youth Empowerment for Island Sustainability. (University of Guam, Mangilao, Guam.). Presented to students participating in the Guam Humanities Project: Taking Root: Growing Youth Empowerment for Island Sustainability at University of Guam, Mangilao, Guam. Sept. 22, 2018. URL: https://ndownloader.figshare.com/files/13141172.

[2] Aubrey Moore. "Coconut Rhinoceros Beetle Causes an Ecological Catastrophe on Guam". In: Presentation for the University of Guam Extension Internship Program. June 21, 2019. URL: https://github.com/aubreymoore/2019-Extension-Internship-CRB-Presentation/raw/master/2019-CRB-catastrophy-presentation.pdf.

[3] Aubrey Moore. "Failed Attempts to Establish IPM for Asian Cycad Scale and Coconut Rhinoceros Beetle on Guam". In: Annual Meeting of the Entomological Society of America. (Vancouver, Canada). Nov. 13, 2018. DOI: 10.5281/zenodo.2545065. URL: https://zenodo.org/record/2545065/files/Moore-Vancouver-2018.pdf (visited on 01/21/2019).

[4] Aubrey Moore. "Impact of Climate Change on Coconut Rhinoceros Beetle Outbreaks in the Pacific". In: Guam Extension and Outreach Climate Forum. (Guam). Oct. 26, 2017. URL: https://github.com/aubreymoore/crb-climate-change/raw/master/crb-climate-connection.pdf.

[5] Aubrey Moore. "Special Report for Guam Invasive Species Awareness Week: Invasive Species Are a Crisis for Guam and the Pacific, Right Now". In: *Pacific Island Times* (Feb. 25, 2018). URL: https://www.pacificislandtimes.com/single-post/2018/02/25/Special-Report-Invasive-species-are-a-crisis-for-Guam-and-the-Pacific-right-now (visited on 08/25/2018).

6 Regional Collaboration

An informal collaboration has been formed among Pacific-based entomologists working on the CRB-G problem. Scientists from Guam, Hawaii, Palau, Papua New Guinea, Solomon Islands, Fiji, Malaysia, Japan and New Zealand have met several times and future meetings are planned:

- 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 (IPPC2019), India
- 2020 (tentative): Pacific Plant Protection Organization, Guam

Although individual institutions involved in finding a solution to the CRB-G problem have secured funding from multiple, short-term grants, we have been unsuccessful in finding funding to support a well-coordinated regional project. To facilitate informal communication and cooperation among members of the current *ad hoc* group, we have spent some time and resources on developing the following resources.

6.1 Wiki Site

During 2018, we built a private wiki intended to facilitate sharing technical information among those working to control CRB-G [4]. It is hoped that this site will be built and maintained by a community of users (ala Wikipedia).

6.2 Facebook Site

As an alternative to the wiki, we are launching a controlled-access Facebook site [9].

6.3 CRB Bibliography

The Guam CRB Biocontrol Project maintains a publicly available bibliography which contains over 330 scientific journal articles, technical reports, and presentations about coconut rhinoceros beetle [8].

6.4 Online CRB Invasion History Map

Spread of CRB and CRB-G in the Pacific is being documented using an online, interactive map maintained by Moore [6]. Recent geographical expansions include CRB (not the CRB-G biotype) in Vanuata, interception of CRB-G in New Caledonia, and discovery of a single CRB-? adult on Aguiguan Island, a small island near Tinian in the Commonwealth of the Northern Mariana Islands.

7 References

[1] Sean D. G. Marshall, Aubrey Moore, Maclean Vaqalo, Alasdair Noble, and Trevor A. Jackson. "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 (Oct. 1, 2017), pp. 127–134. ISSN: 0022-2011. DOI: 10.1016/j.jip.2017.07.006. URL: http://www.sciencedirect.com/science/article/pii/S0022201117300289 (visited on 08/26/2017).

[2] Aubrey Moore. *Additional Documentation in Support of a Permit Application for Importation of Live Coconut Rhinoceros Beetles to Guam*. June 2, 2019. URL: https://github.com/aubreymoore/CRB-Import-Permit/raw/master/CRB-import-permit-request-additional.pdf.

[3] Aubrey Moore. *Coconut Rhinoceros Beetle Rearing Database*. Oct. 13, 2019. URL: http://aubreymoore.pythonanywhere.com/rearing.

[4] Aubrey Moore. *CRB-G Wiki*. Oct. 13, 2019. URL: http://guaminsects.net/CRBG/index.php?title=CRB-G_Wiki.

[5] Aubrey Moore. *Open Science Framework: Digital Image Analysis for Detection of CRB Damage in Videos*. Oct. 12, 2019. URL: https://osf.io/czr8t/?view_only=cdec7cc47b514b31b33115113193a2d4.

[6] Aubrey Moore. *Web Page: Online Interactive Map of Coconut Rhinoceros Beetle Invasion History*. 2019. URL: http://aubreymoore.github.io/crbdist/mymap.html (visited on 05/28/2019).

[7] Aubrey Moore. *YouTube: Training an Object Detector to Locate Coconut Palms Damaged or Killed by Coconut Rhinoceros Beetle*. Oct. 9, 2019. URL: https://www.youtube.com/watch?v=zzSorqcmt9U.

[8] Aubrey Moore and James Grasela. *Coconut Rhinoceros Beetle Bibliography*. 2019. URL: https://github.com/aubreymoore/CRB-Bibliography.

[9] Aubrey Moore and Ian R. Iriarte. *FaceBook Site: CRB-G*. Oct. 13, 2019. URL: https://www.facebook.com/groups/crbg07/.

[10] Aubrey Moore and Roland Quitugua. *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, June 1, 2017, p. 3. URL: https://github.com/aubreymoore/CRB-Import-Permit/raw/master/CRB_Shipping_Container.pdf.

[11] USDA-APHIS. *CRB Import Permit*. July 5, 2019. URL: https://github.com/aubreymoore/CRB-Import-Permit/raw/master/2019-CRB-Import-Permit.pdf.

[12] Shizu Watanabe and Michael Melzer. "Survey for Oryctes Rhinoceros Nudivirus (OrNV) in a Hawaiian coconut Rhinoceros Beetle (Oryctes Rhinoceros) Population and genetic Diversity of Pacific isolates of OrNV". In: 49th Annual Meeting of the Society for Invertebrate Pathology. (Tours, France). 2016. URL: http://www.sipweb.org/docs/Abstract_book_SIP_2016.pdf (visited on 07/31/2018).