Biological Control of Coconut Rhinoceros Beetle Biotype G

Aubrey Moore, University of Guam

July 26, 2017

Contents

A newly discovered biotype of coconut rhinoceros beetle (CRB-G) is rapidly killing coconuts and other palms on Guam. Following a failed eradication attempt, CRB-G proved hard to control because it is resistant to Oryctes nudivirus (OrNV), which was previously used as the preferred biological control agent for control of CRB outbreaks on Pacific Islands and elsewhere.

The overall objective of this project is to stop the uncontrolled outbreak on Guam. Pacific-based entomologists working on this problem agree that the most feasible solution is to find a new isolate of OrNV which is highly pathogenic to CRB-G. Foreign exploration has already discovered an OrNV isolate from an infected CRB-G collected in the Philippines. If laboratory bioassays indicate that this isolate is pathogenic for CRB-G, it will be propogated and distributed throughout Guam by autodissemination. All previous OrNV releases on Pacific Islands resulted in immediate and sustained suppression of CRB damage to low levels and prevented tree mortality. We hope to find an OrNV isolate which will produce similar results.

Guam is currently experiencing an uncontrolled and unmonitored island-wide CRB-G outbreak which was triggered by abundant CRB-G breeding sites in the form of dead and dying vegetation left in the wake Typhoon Dolphin which occured in May 2015. of a recent typhoon. Most of these breeding sites are inaccessable to sanitation efforts, being either in the jungle or on military land (which covers one third of Guam). A positive feedback cycle has begun whereby large numbers of adult beetles are killing large numbers of palms which become breeding sites which generate even higher numbers of adults. Severe damage to Guam's palms prompted the Governor of Guam to declared a state of emergency in July 2017.

Entomologists working on this problem agree that the most feasible tactic to halt tree mortality and suppress damage to tolerable levels is establishment of biological control using an isolate of OrNV which is highly pathogenic to CRB-G. Concurrent with establishment of CRB-G biocontrol, success of the project will be monitored in a quarterly, island-wide tree health survey and incidence of OrNV infection will be monitored in a subsample of all field collected CRB-G.

If the Guam CRB-G infestation cannot be controlled, it is expected that most palms on the island will be killed and CRB-G will spread to other islands and beyond. If CRB-G invades smaller islands and atolls where coconut is *the tree of life*, a human tragedy will ensue. On larger islands, coconut and oil palm industries will be severely impacted. Attempts to organize a regional project in response to CRB-G are underway.

1 Technical Approach

Coconut rhinoceros beetle (CRB), *Oryctes rhinoceros*, is a major pest of palms. Adults bore into crowns to feed on sap. A palms maybe killed if CRB feeding activity damages the meristem. But this rarely happens at low CRB population densities. CRB grubs do no damage. They feed on decaying vegetation with standing dead coconuts and fallen coconut logs being favored sites. In addition, they can feed in many type of organic matter including dead trees, green waste, saw dust, manure, compost, and even in bags of commercially packaged soil [1].

CRB was first detected on Guam in 2007. Following failure of an eradication attempt using mass trapping and sanitation, the beetle spread to all parts of the island within a few years. *Oryctes* nudivirus (OrNV) and green muscardine fungus (GMF), *Metarhizium majus*, where introduced as biological control agents. GMF was successfully established and a 2015 survey indicated that between 10% and 38% of Guam's CRB were infected by this fungus [2]. However, the preferred biocontrol agent for CRB, namely OrNV failed to have any effect. Prior to the OrNV biocontrol failure on Guam, releases on other Pacific islands resulted in reduction in CRB damage by up to 90% within a few moths with control lasting for 30 plus years [3].

This lead us to discover that the population of coconut rhinoceros beetles (CRB) recently established on Guam is genetically distinct from other populations of this major palm pest and it is being referred to as the CRB-G biotype [4, 5]. CRB-G is resistant to all available isolates of OrNV, previously the most effective biocontrol agent for CRB, and it appears to have other characteristics, which make it more invasive and harder to control than other CRB biotypes. While there where no range expansions of CRB for a quarter of a century (1980 to 2005), CRB is now on the move with the invasion of Guam in 2007, the Port Moresby area of Papua New Guinea in 2009, Oahu, Hawaii in 2013, and the Honiara area of Guadalcanal, Solomon Islands in 2015. It is significant that all of these new invasions involve CRB-G. Thus, CRB-G is a regional problem which poses significant risks to Pacific island economies and ecosystems. Concerned Pacific-based entomologists are attempting to raise support for coordinated regional response to CRB-G [6, 7, 8]. APHIS supported this effort by hosting a meeting at the International Congress of Entomology, Florida, 2016 [9].

Guam is currently experiencing massive mortality of coconut palms as the result of a CRB population explosion triggered by abundant larval breeding sites left in the wake of a Typhoon Dolphin which visited the island in May 2015. Many coconut palms were killed by numerous adults emerging from decaying vegetation, most of which was inaccessible to sanitation, being in the jungle and/or on military land. Resulting dead, standing coconuts quickly turned into breeding sites which are generating even higher numbers of CRB adults. This type of positive feed back cycle has been observed elsewhere following tropical storm damage, large-scale land clearing or war. An CRB outbreak occurred in the Palau Islands immediately following WWII resulting in about 50% coconut palms being killed by CRB throughout the archipelago and 100% mortality on some of the smaller islands [10]. A similar outcome can be expected for Guam if the current outbreak is not controlled. In addition spread of CRB-G to other islands and beyond can be expected.

CRB has already been intercepted on Saipan Island, about 200 miles north of Guam. Severity of the Guam CRB-G outbreak resulted in Guam's Governor Calvo declaring a state of emergency in July 2017.

The overall objective of this project is to stop an uncontrolled and unmonitored outbreak of CRB-G on Guam which is rapidly killing coconut and and other palms. Entomologists working on this problem agree that the most feasible solution is establishment of biological control using an isolate of OrNV which is highly effective for CRB-G [6, 7, 8].

Financial assistance will facilitate:

1. hiring a post-doc entomologist to assist with this project and continued support for a graduate research assistant at the University of Guam
2. continued support for operating an insect pathology laboratory at the University of Guam to evaluate and propagate candidate biocontrol agents discovered during foreign exploration
3. support for a quarterly island-wide coconut palm health survey for Guam
4. continued support for organizing an international collaborative project with the goal of discovering a strain of OrNV or other microbial biocontrol agent which is highly pathogenic for CRB-G

This project is aligned with FB Goal 6: *Enhance Mitigation and Rapid Response* and it builds on progress made with the support of FB funds from FY2014 through FY2017.

1.1 Objective 1: Find an OrNV Isolate which is Highly Pathogenic for CRB-G

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

During January, 2017, Moore, Iriarte and Marshall did field work on Negros Island, Philippines, were CRB-G coexists with other CRB biotypes. The major objective was to find an effective biocontrol agent for CRB-G and a secondary objective was to develop and test protocols for further foreign exploration. DNA analysis of CRB and OrNV from rhino beetle gut samples collected during the trip is being done by Dr. Sean Marshall in his lab at AgResearch New Zealand. Bioassays of any detected OrNV will be done at the University of Guam. Further foreign exploration for an effective biocontrol agent for CRB-G is contingent on results from this first expedition. A report of the Negros expedition is included in the Prior Experience section.

1.1.2 Regional Collaboration on CRB-G Management

Moore will continue to work with collaborators at AgResearch New Zealand, the Secretariat of the Pacific Community (SPC), and USDA-APHIS to put together a regional collaboration with the objective of finding an effective biocontrol agent for CRB-G.

1.2 Objective 2: Establish Sustainable Biocontrol of CRB-G by Autodissemination

If the OrNV isolate from the infected beetle collected on Negros Island, Philippines does not prove to be pathogenic for Guam beetles in a high dose lab bioassay, foreign exploration will continue until a pathogenic isolate is found. Otherwise, introduction of the virus into the Guam CRB population via auto-dissemination will commence. Autodissemination is a proven method for rapid establishment of OrNV as a self sustaining biocontrol agent in CRB populations. Healthy CRB adults are dosed with OrNV and released from multiple points. Before these beetles get sick, they spread the virus within the healthy population during mating and visits to breeding sites.

OrNV will be propagated *in vitro* (in insect cell culture) at AgResearch New Zealand and also *in vivo* (in live CRB-G) at the University of Guam. Beetles for auto-dissemination will be field collected from breeding sites and pheromone traps because this is far more efficient than rearing beetles in the lab at the current time. A subsample of field collected beetles will dissected for visual detection of OrNV infection, and gut tissue will be preserved for molecular testing at AgResearch.

Concurrent with auto-dissemination releases, laboratory bioassays will be performed to quantify the toxic (LD50, LT50, etc.) and nontoxic effects (fecundity, flight capability, etc.) of OrNV on CRB. There will also be an attempt to increase virulence by cycling isolates through several generations of beetles. Beetles used in bioassays will be field collected and maintained in individual Mason jars for at least a month prior to being used to make sure they are healthy. Beetles used for auto-dissemination will flight tested an marked with a unique number prior to release using the methods outlined in Moore et al. 2017 [11].

1.3 Objective 3: 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. We intend to establish a quarterly coconut tree health survey rather than re-establish pheromone trapping.

1.3.1 Survey Method

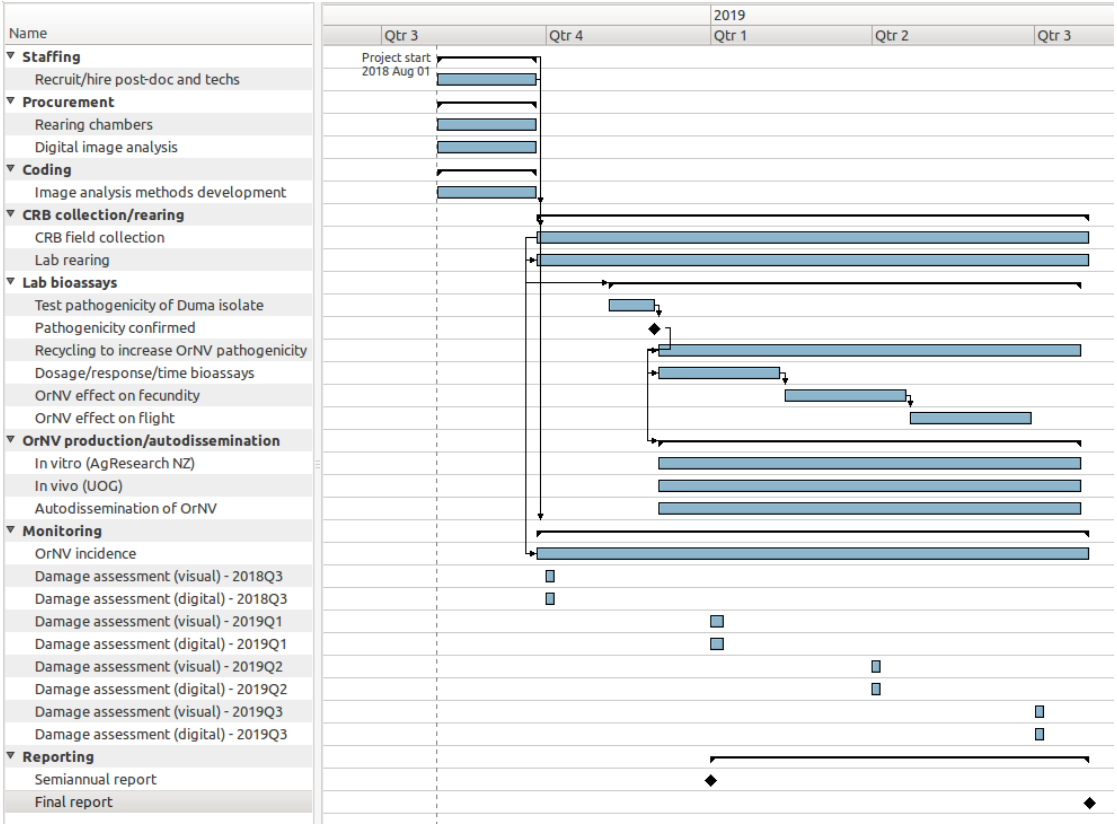
The Coconut Palm Health Survey will use the following methodology to track changes in levels of damage caused by CRB-G.

- The survey will monitor at least 1,000 palms located throughout the island. An aluminum tag with a unique identifier will be affixed to each palm on the initial visit.
- The free smart phone app, EpiCollect+ will be used to georeference each palm, record a digital image, and record damage data. (We have successfully used this free app for several localized palm health surveys.)
- The survey will be performed four times per year.
- CRB damage will be recorded in 3 boolean data fields:
  - Mortality: 1 if palm is dead; 0 otherwise
  - New damage: 1 if any of the 4 youngest fronds have V-shaped cuts; 0 otherwise
  - Old damage: 1 if any other fronds have V-shaped cuts; 0 otherwise

1.3.2 Digital Image Analysis

We propose to add a methods development component to the survey. CRB damage symptoms in the form of V-shaped cuts in fronds are distinctive and easy to see in digital images. Digital imagery has been used for detection and monitoring of CRB. For example, Soloman Sar in Papua New Guinea has developed a Rapid Damage Assessment System in which geotagged images of palms are rated for damage severity. It may be possible to automate detection and monitoring of CRB damage by training a computer to detect V-shaped cuts in digital images of coconut palms. We will test this idea using human classified image libraries as training sets. If successful, we will program a Raspberry Pi 3 equipped with a camera to detect and quantify CRB damage in real time. This small, inexpensive CRB damage detector could be mounted on a drone or a conventional vehicle for automated detection and monitoring of CRB and damage caused by this pest.

1.4 Timeline


Name
2019
Qtr 3
Qtr 4
Qtr 1
Qtr 2
Qtr 3
Project start
2018 Aug 01
Staffing
Recruit/hire post-doc and techs
Procurement
Rearing chambers
Digital image analysis
Coding
Image analysis methods development
CRB collection/rearing
CRB field collection
Lab rearing
Lab bioassays
Test pathogenicity of Duma isolate
Pathogenicity confirmed
Recycling to increase OrNV pathogenicity
Dosage/response/time bioassays
OrNV effect on fecundity
OrNV effect on flight
OrNV production/autodissemination
In vitro (AgResearch NZ)
In vivo (UOG)
Autodissemination of OrNV
Monitoring
OrNV incidence
Damage assessment (visual) - 2018Q3
Damage assessment (digital) - 2018Q3
Damage assessment (visual) - 2019Q1
Damage assessment (digital) - 2019Q1
Damage assessment (visual) - 2019Q2
Damage assessment (digital) - 2019Q2
Damage assessment (visual) - 2019Q3
Damage assessment (digital) - 2019Q3
Reporting
Semiannual report
Final report

References

[1] A. Moore, R. Quitugua, I. Iriarte, M. Melzer, S. Watanabe, Z. Cheng, and J. M. Barnes, "Movement of Packaged Soil Products as a Dispersal Pathway for Coconut Rhinoceros Beetle, Oryctes rhinoceros (Coleoptera: Scarabaeidae) and Other Invasive Species," *Proceedings of the Hawaiian Entomological Society*, vol. 48, pp. 21–22, Dec. 2016. [Online]. Available: http://scholarspace.manoa.hawaii.edu/handle/10125/42743

[2] A. Moore and S. Marshall, "Efficacy of Entomopathogenic Fungus for Biological Control of Coconut Rhinoceros Beetle (CRB) on Guam and DNA Profiling of Asia/Pacific CRB Populations with Respect to Virus Susceptibility," 2015. [Online]. Available: http://guaminsects.net/anr/sites/default/files/semiannual-report-April2015.pdf

[3] G. O. Bedford, "Long-term reduction in damage by rhinoceros beetle Oryctes rhinoceros (L.) (Coleoptera: Scarabaeidae: Dynastinae) to coconut palms at Oryctes Nudivirus release sites on Viti Levu, Fiji," *African Journal of Agricultural Research*, vol. 8, no. 49, pp. 6422–6425, Dec. 2013. [Online]. Available: http://www.academicjournals.org/journal/AJAR/article-abstract/57F27A342330

[4] S. D. G. Marshall, M. Vaqalo, A. Moore, R. Quitugua, and T. A. Jackson, "A new invasive biotype of the coconut rhinoceros beetle (Oryctes rhinoceros) has escaped from biocontrol by Oryctes rhinoceros nudivirus," in *International Congress on Invertebrate Pathology and Microbial Control and the 48th Annual Meeting of the Society for Invertebrate Pathology*, 2015. [Online]. Available: http://www.sipmeeting.org/van1/SIP2015-FullProgram.pdf

[5] S. D. G. Marshall, A. Moore, M. Vaqalo, 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 preparation]," *Journal of Invertebrate Pathology*, 2017.

[6] T. A. Jackson, "NEED FOR EMERGENCY RESPONSE FOR A NEW VARIANT OF RHINOCEROS BEETLE (GUAM BIOTYPE," *International Association for the Plant Protection Sciences Newsletter*, no. XI, Nov. 2015. [Online]. Available: https://www.plantprotection.org/portals/0/documents/newsletters/2015/iapps%2011-2015.pdf

[7] M. Vaqalo, S. Marshall, T. Jackson, and A. Moore, "An emerging biotype of coconut rhinoceros beetle discovered in the Pacific," Secretariat of the Pacific Community, Pest Alert 51, 2015. [Online]. Available: http://westernipm.org/index.cfm/center-projects/signature-programs/invasive-species/coconut-rhinoceros-beetle/pest-alert-coconut-rhino-beetle-final-pdf/

[8] S. D. G. Marshall, A. Moore, and M. Vaqalo, "White Paper: A New Coconut Rhinoceros Beetle Biotype Threatens Coconut and

Oil Palms in Southeast Asia and the Pacific," Jul. 2016. [Online]. Available: http://westernipm.org/index.cfm/center-projects/signature-programs/invasive-species/coconut-rhinoceros-beetle-pdf/

[9] A. Moore, "Meeting to Discuss a Response to CRB-G, International Congress of Entomology, Orlando, Florida, Meeting in Orlando, Florida, September 2016," 2016. [Online]. Available: https://osf.io/67g2m/

[10] L. J. Gressitt, "The coconut rhinoceros beetle (Oryctes rhinoceros) with particular reference to the Palau Islands," Honolulu, Tech. Rep., 1953. [Online]. Available: https://books.google.com/books?id=OtcZAQAAIAAJ

[11] A. Moore, D. C. Barahona, K. A. Lehman, D. A. Skabeikis, I. R. Iriarte, E. B. Jang, and M. S. Siderhurst, "Judas beetles: Discovering cryptic breeding sites by radio-tracking coconut rhinoceros beetles, Oryctes rhinoceros (Coleoptera: Scarabaeidae)," *Journal of Environmental Entomology*, vol. 46, no. 1, pp. 92–99, 2017.

2 Impacts and Benefits

- Foreign exploration leading to discovery of a highly pathogenic strain of OrNV or other microbial biocontrol agent for CRB-Guam could lead to implementation of self sustaining population suppression and tolerable damage levels on Guam and other islands invaded by CRB-G.
- Loss of 50% or more of Guam's palms may be prevented if an effective biocontrol agent is found and released quickly.
- Reduction in CRB population levels on Guam will reduce the risk of accidental of the highly invasive CRB-Guam biotype to other Pacific islands and elsewhere.
- Development of image analysis methods may lead to a small, inexpensive, automated CRB damage detector which could be mounted on a drone or a conventional vehicle. This device could be used for early detection or monitoring of CRB damage.