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.

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 atols where coconut is *the tree of life*, a human tragedy will ensue. On larger islands, coconut and oil palm industries will be severely impacted.

Guam is currently experiencing an uncontrolled and unmonitored CRB-G outbreak which was triggered by abundant CRB-G breeding created by a typhoon. 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 solution is establishment of biological control using an isolate of OrNV which is highly pathogenic to CRB-G

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 prefered biocontrol agent for CRB, namely OrNV failed to have any effect. 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[3, 4]. 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[5, 6, 7]. APHIS supported this effort by hosting a meeting at the International Congress of Entomology, Florida, 2016.

, with Guam 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 recent typhoon.

The overall objective of this project is to stop an uncontrolled 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 pathogenic to CRB-G [REFERENCES].

Financial assistance will facilitate:

1. continued support of 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

2. hiring a post-doc entomologist to assist with this project and continued support for a graduate research assistant at the University of Guam

3. continued support for operating an insect pathology laboratory at the University of Guam to evaluate candidate biocontrol agents discovered during foreign exploration

4. support for a semiannual island-wide coconut palm health survey for Guam

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 Regional Collaboration on CRB-G Management

Moore will continue to work with collaborators at AgResearch New Zealand and the Secretariat of the Pacific Community (SPC) to put together a regional collaboration

with the objective of finding an effective biocontrol agent for CRB-G.

1.1.2 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.

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 proves to be pathogenic for Guam beetles in a high dose lab bioassay, introduction of the virus into the Guam CRB population via autodissemination will commence. Otherwise, foreign exploration will continue until a pathogenic isolate is found.

If the OrNV Negros isolate proves to be pathogenic for CRB-G, in vitro production in insect cell culture will be ramped up at AgResearch lab in New Zealand. 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. On Guam, beetles for autodissemination 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.

Concurrent with autodissemination 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.

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 semiannual coconut tree health survey rather than re-establish pheromone trapping.

1.3.1 Survey Method

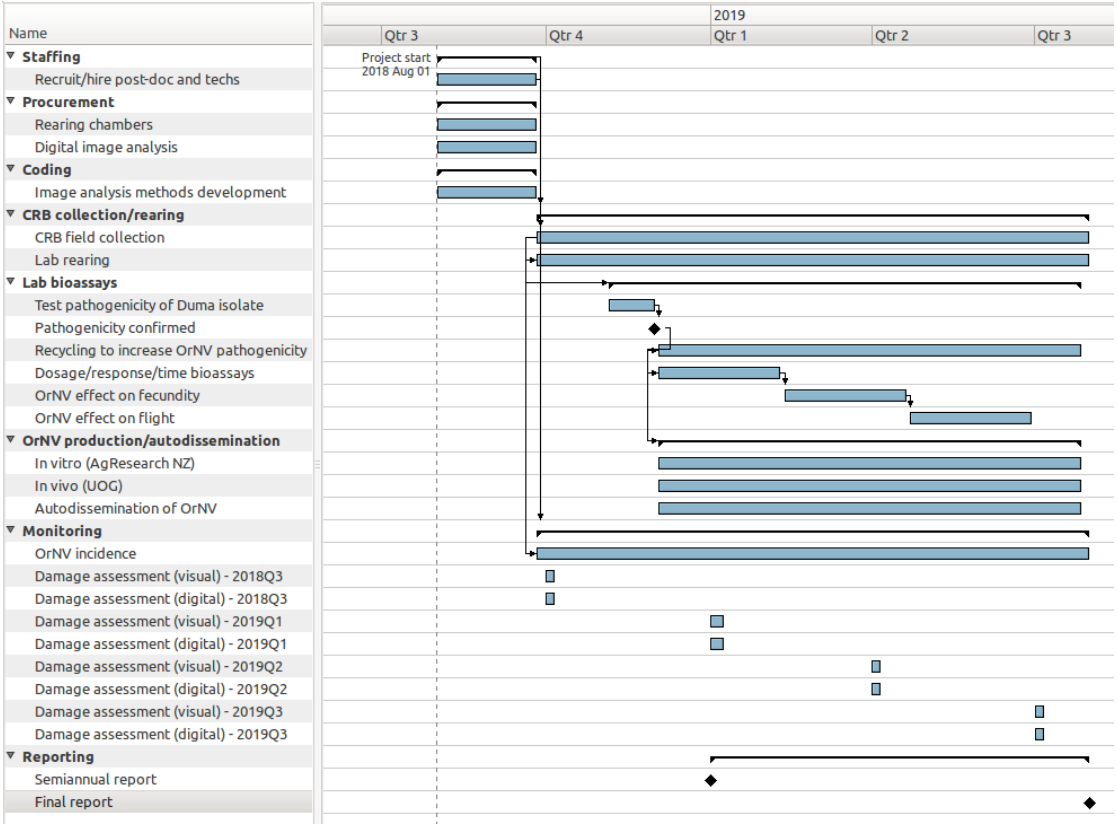
The Coconut Palm Health Survey will use the following methodology to track changes in levels of damage caused by CRB-G.

- 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 twice 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



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] 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

[4] 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.

[5] 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

[6] 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/

[7] 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/

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 Pacifc 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.

3 Prior Experience

3.1 Most Recent APHIS Farm Bill Progress Report, January 2017

Please see following page.

Oryctes Nudivirus for Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle

Aubrey Moore, University of Guam

January 19, 2017

Year: FY2016

State: Guam

Cooperative Agreement Name: Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle

Cooperative Agreement Number: 16-8515-2058-CA

Project Funding Period: August 1, 2016 through July 30, 2017

Project Report: Farm Bill Survey Report

Project Document Date: July 12, 2016

Cooperators Project Coordinator: Aubrey Moore

Name: Aubrey Moore

Agency: College of Natural and Applied Sciences, University of Guam

Address: 303 Campus Drive

City/Address/Zip: Mangilao, Guam 96923

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Type of Report: Semi-Annual Accomplishment Report

Performance period covered by this report: August 1, 2016 through December 31, 2016

Contents

1 Objectives and Need for Assistance

The abstract from the Farm Bill Suggestion for this project provides a useful introduction:

> 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. CRB-G is resistant to Oryctes nudivirus, which is the major 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.
>
> This FB suggestion is a request for funding to be used as seed money to organize an international collaborative project with the goal of discovering a strain of OrNV or other microbial biocontrol agent which is highly pathogenic for CRB-Guam, to hire a graduate research assistant and to establish a insect pathology laboratory on Guam to evaluate candidate biocontrol agents discovered during foreign exploration.

1.1 Urgent Need to Mitigate Mature Palm Mortality Caused by CRB-G on Guam

Mortality of mature palms has increased dramatically over the past year as a result of abundant new breeding sites in the form of decaying vegetation left in the wake of Typhoon Dolphin which visited Guam in May 2015. It appears that the typhoon triggered a positive feedback cycle where CRB adults are numerous enough to large numbers of mature palms. The resulting dead standing coconut stems become optimum breeding sites which produce even higher numbers of adults. Uncontrolled outbreaks such as this occurred in Palau and Fiji, resulting in coconut palm mortality of 50% or more. Current tactics of trapping, sanitation, and application of Metarhizium may reduce local damage but are ineffective in preventing wide-spread island-wide damage because most breeding sites are inaccessible, in jungle and/or on military bases.

There is an urgent need to find and release an effective isolate of OrNV, or another effective density-dependent biocontrol agent, for CRB-G. Without a rapid response, most of Guam's palms may be killed and the risk of accidental transport of CRB-G to other islands is high. If CRB-G invades atolls where coconut is still "the tree of life" or islands where coconut and/or oil palms are major crops this could lead to a humanitarian disaster.

1.2 Need for Regional Collaboration to Manage CRB-G on Pacific Islands

The CRB-G biotype issue is a new emergent pest problem that has Pacific islands entomologists very worried.

- Sean Marshall presented a report entitled "A new invasive biotype of the coconut rhinoceros beetle (Oryctes rhinoceros) has escaped from biological control by Oryctes rhinoceros nudivirus" at the International Congress on Invertebrate Pathology and Microbial Control and the 48th Annual Meeting of the Society for Invertebrate Pathology in Vancouver, BC, Canada on August 13, 2015.

- The University of Guam published a press release entitled Pacific Entomologists are Worried About a New Type of Coconut Rhinoceros Beetle Discovered on Guam on September 2, 2015. This press release describes the rapidly worsening damage caused by CRB on Guam.

- Trevor Jackson published a note entitled Need for emergency response for a new variant of rhinoceros beetle (Guam biotype) in the current edition (Nov. 2015) International Association for the Plant Protection Sciences Newsletter. In this note, Jackson suggests the following steps should be taken as soon as possible to avert large scale ecological and economic damage to palms by rhino beetle invasions on Pacific islands:

    1. Raise awareness through biosecurity networks of the potential threat of CRB-Guam and provide information for early detection and eradication of limited outbreaks
    2. Form an International Working Group to develop a strategy for control and containment and coordinate activities.
    3. Identify funding sources and secure funding for key participating institutes.
    4. Carry out a thorough delimiting survey to identify current distribution of CRB-G and identify center of origin.
    5. Find and test Oryctes nudivirus variants to find CRB pathogenic strains.
    6. Implement control and containment strategy to limit impact and spread of the beetle.

- The Pacific Plant Protection Organization (PPPO) met in Fiji during the week of September 21, 2015, attended by reps from 22 Pacific Island countries and territories, the Secretariat of the Pacific Community, AgResearch New Zealand, United Nations Food and Agriculture Organization, and the United States Department of Agriculture, and federal governments of Australia and New Zealand. CRB-G was discussed at length. Jackson's suggestions were endorsed by the PPPO and the Pacific Community (SPC) was asked to assist in formulating plans and finding funding for a regional collaboration to implement these suggestions. In addition, it was suggested that "Exploration of effective biological control candidates, especially virus from the native range of the CRB-G biotype." should be a priority action item. Although success in finding an effective OrNV isolate is not guaranteed, experts suggest there is a high probability of finding such an isolate infecting beetles near the origin of the CRB-G biotype. An endemic population of CRB-G has been found on Negros Island in the Philippines. In addition, Palau has CRB-G and other CRB biotypes. This grant will support a two weeks of field work by Moore, Marshall, and Iriarte in Palau and the Philippines. The

major objective is to find an effective biocontrol agent for CRB-G and a secondary objective is to develop and test protocols for further foreign exploration.

- SPC sponsored a workshop in Fiji during June 1-3, 2016. A half-day session during this workshop was on the topic "Developing a response to the threat of CRB-G. Information exchange, development of response plans, coordination and development of new projects."

- The next opportunity for a face-to-face meeting of entomologists working on CRB-G will be at the International Congress of Entomology in Orlando, Florida during September, 2016. Many of those involved will participate in a symposium organized by Trevor Jackson and Mike Kline entitled "Scarabs without Borders: Lessons from a Century of Invasions". Plans are to use this event as an opportunity to organize a regional collaboration. This grant will support Aubrey Moore's participation in this symposium. He will make a presentation entitled "The Rhinoceros Beetle Invasion of Guam: An Unprecedented Disaster".

2 Results or Benefits Expected

- 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.

- 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 transport of the highly invasive CRB-Guam biotype to other Pacific islands and elsewhere An effective biocontrol agent for Guam's CRB infestation will be useful against CRB-Guam invasions elsewhere.

3 Approach

3.1 "Witch's Brew" Bioassays

In previous years, we tested several isolates of OrNV from AgResearch New Zealand and some from virus-infected beetles in Fiji. We did not observe significant mortality during many bioassays, leading us to the conclusion that CRB-G is resistant to OrNV. However, to confirm that we do not have OrNV pathogenic for CRB-G, we have started a series of "witch's brew" bioassays. Frozen, dead beetles from all previous bioassays were added to one liter of water and made into an aqueous slurry using a blender. Vials containing remnants of virus samples from AgResearch New Zealand were agitated in 500 ml of water, and this suspension was added to the blender. The slurry was poured into a small pail and forty beetles were made to swim in this for thirty minutes. A control group of beetles was made to swim in water for thirty minutes. Beetles were kept in a large container filled with moist,

commercially blended steer manure and soil. All beetles were checked weekly. Dead beetles were recorded and frozen.

We found a significantly higher mortality in beetles which swam in the slurry as opposed to beetles which swam in water. We made a fresh "witch's brew" by blending all dead beetles from this assay, and again observed mortality significantly higher than that of the control group. We will continue these witch's brew experiments and send beetle tissue samples to AgResearch New Zealand to test for OrNV.

3.1.1 Progress

Technical Support and Collaboration

- As per the work plan, a graduate student was recruited as a research assistant for this project. Ian Iriarte will fill this role as he earns a masters degree in the University of Guam's Environmental Science program. Mr. Iriarte's research topic is "Biological Control of Coconut Rhinoceros Beetle".

- A contract was prepared to facilitate collaboration between UOG and AgResearch New Zealand. Dr. Sean Marshall and Dr. Trevor Jackson, world experts on biocontrol of CRB using OrNV, work for this research center. The contract has been signed by Agresearch New Zealand and it is currently being circulated for signatures at UOG. Collabortation with colleagues at AgResearch is essential to this project because they have the skills and facilities to detect OrNV, genotype CRB, and propogate OrNV. Molecular diagnostics of a backlog of specimens in UOG freezers is awaiting completion of the contract.

Witch's Brew Experiment

- We have now completed 4 iterations of the "witch's brew" experiment. Results are summarized in table 1 and details are in tech reports available online at:

  https://github.com/aubreymoore/Witch-s-Brew/blob/master/witchesBrew1/wb1.pdf
  https://github.com/aubreymoore/Witch-s-Brew/blob/master/witchesBrew2/wb2.pdf
  https://github.com/aubreymoore/Witch-s-Brew/blob/master/witchesBrew3/wb3.pdf
  https://github.com/aubreymoore/Witch-s-Brew/blob/master/witchesBrew4/wb4.pdf

- Treatment mortality for beetles forced to swim in the "witch's brew" is high and continues to rise with each iteration. However, control mortality is high, about 30-40%, mainly due to the fungal pathogen, *Metarhizium majus*. We have attempted to filter out fungal spores by passing the brew through a serious of filters with the last stage being a Millipore 0.45 micron filter, which should block all fungal spores while allowing virus particles to pass through. Unfortunately, this idea has not worked because filters get totally plugged.

- To date, we have no indication that any of the beetles which died in the "witch's brew" experiment were killed by OrNV. We are awaiting signing of the contract with Ag Research so that we can send samples to Dr. Marshall for virus detection. If any virus

Table 1: Mortality of beetles forced to swim in the witch's brew. **Treatment mortality** is corrected for control mortality using Abbott's formula. **p** is the probability of treatment mortality exceeding control mortality by chance (Fisher's exact test).

iteration	treatment mortality	p
1	51%	0.0005
2	53%	0.0014
3	82%	0.0000
4	84%	0.0000

is detected, we will resume iterations of the "witch's brew" experiment in an attempt to increase virulence.

- We have also trapped a series of beetles an dissected out guts to send to Ag Research for virus detection. Biological control agents often arrive a few years after detection of invasive species. These "fortuitous introductions" are common. Samples will be sent to Dr. Marshall for virus detection when the AgResearch contract is signed.

3.2 Regional Collaboration on CRB-G Management

Moore will continue work with collaborators at AgResearch New Zealand and SPC to put together a regional collaboration with the objective of finding an effective biocontrol agent for CRB-G. Plans will be developed and moved forward at the scarab beetle symposium at the International Symposium of Entomology.

3.2.1 Progress

Activities at the International Congress of Entomology, Orlando, Florida

- Participated in a symposium entitled Scarabs without **Borders: Lessons from a Century of Invasions**. Abstracts for presentations relevent to CRB are available at: https://aubreymoore.github.io/CRB-G-ICE2016/Session26139.html.
  - Delivered an invited oral presentation: **The rhinoceros beetle invasion of Guam: An unprecedented disaster.** Aubrey Moore, University of Guam; Roland Quitugua, University of Guam; Trevor Jackson, AgResearch Ltd; Sean Marshall, AgResearch Ltd; Matthew Siderhurst, Eastern Mennonite University
  - Co-authored presentation: **Detection of an invasive biotype of Oryctes rhinoceros (L.) in the Pacific.** Sean Marshall, AgResearch Ltd; Maclean Vaqalo, Secretariat of the Pacific Community; Aubrey Moore, University of Guam; Roland Quitugua, University of Guam; Trevor Jackson, AgResearch Ltd
- I helped to organized and participated in a special meeting to discuss a regional response to the coconut rhinoceros beetle biotype. This meeting was sponsored by USDA-APHIS and co-chaired by Dr. Ron Weeks and Philipp Andreozzi. Minutes from this

meeting and associated data are saved in an Open Science Framework project I created at https://osf.io/67g2m/. A press release about the meeting including a photo of participants can be found here: https://osf.io/qsd8p/.

Collaboration with University of Hawaii

- On my way back from the ICE, I met with colleagues at UH-Manoa to discuss and participate in coconut rhinoceros beetle research: Mike Melzer, Shizu Watanabe, Zhiqiang Cheng, Dan Jenkins, John Allen, Hans Ramm, Mitch McLean. Discussed collaboration on development of instrumentation. Photos from a lab session available here: https://flic.kr/s/aHskK1bjRC.

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

Early in 2017, Moore, Iriarte and Marshall will do field work in Palau and the Philippines. The major objective is to find an effective biocontrol agent for CRB-G and a secondary objective is to develop and test protocols for further foreign exploration. DNA analysis of CRB and OrNV will be done by AgResearch. Bioassays of any detected OrNV will be done at the University of Guam.

3.3.1 Progress

- As per the work plan, we were to visit both the Philippines and Palau during our initial foreign exploration to prospect for an OrNV isolate which can be used as an effective biocontrol agent for CRB-G. We have decided to skip a visit to Palau because these islands have been recently surveyed by our UH colleagues and we don't want to duplicate their work. Plus, we feel we have a much higher chance of finding OrNV attacking CRB-G in the Philippines which we think is within the native range of CRB-G. We will start our search on Negros Island. CRB previously collected on this island by myself and the Philippine Coconut Authority were all genotyped as CRB-G. Our trip, to include Dr. Sean Marshall, Ian Iriarte, and myself, is planned for January 23 through February 4, 2017.

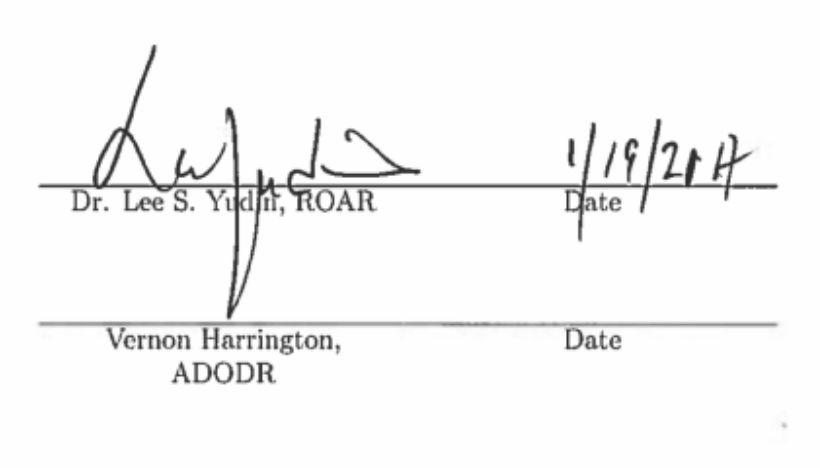
4 Publications

[1] A. Moore, R. Quitugua, I. R. Iriarte, M. Melzer, S. Watanabe, Z. Cheng, and J. Muna-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, 2016. [Online]. Available: http://hdl.handle.net/10125/42743

[2] A. Moore, D. C. Barahona, K. A. Lehman, D. D. 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)," *Environmental Entomology*, 2016. [Online]. Available: http://ee.oxfordjournals.org/content/early/2016/12/05/ee.nvw152

[3] S. D. G. Marshall, A. Moore, M. Vaqalo, and T. A. Jackson, "A new, virus-free haplotype of the coconut rhinoceros beetle (Oryctes rhinoceros) invades the Pacific region [IN PREPARATION]," *Journal of Invertebrate Pathology*, 2017.

5 Signatures



3.2 Report on Foreign Exploration for OrNV in Negros Island, Philippines, January 2017

Please see following page.

Molecular analysis of *Oryctes rhinoceros* collected from Philippines (January 2017)

Sean Marshall and Aubrey Moore

May 2017

Report for University of Guam

Client Report Number: TBD

agresearch

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Molecular analysis of *Oryctes rhinoceros* collected from Philippines (January 2017)

1. EXECUTIVE SUMMARY

Oryctes rhinoceros nudivirus (OrNV) has been effective in reducing *Oryctes rhinoceros* (coconut rhinoceros beetle; CRB) population levels and keeping them at low levels elsewhere in the Pacific for over 40 years. However, to date the CRB population that has invaded Guam (CRB-G haplotype) has so far proven recalcitrant to currently available OrNV isolates that are able to cause disease in other CRB populations. OrNV is known to be widespread across the Philippines, where CRB is considered as native, and the CRB-G haplotype has been identified from here. Native habitats provide a good opportunity to identify candidate OrNV isolates (and other potential biocontrol agents).

The objective of this project was to begin searching for new OrNV isolates that could be effective against the CRB-G haplotype. Identification of an OrNV isolate with good efficacy against CRB-G would assist in preventing further mortality and damage to coconut palms on CRB-G infested Pacific islands (such as Guam), and slow or halt its spread into other areas of the Pacific region.

CRB tissue samples were obtained from live CRB adults collected using both pheromone trapping and hand collection from the Philippines. Gross observation and molecular DNA analyses were used to determine the OrNV infection status and haplotype of individual specimens. The pheromone trapping results were disappointing, with only a single CRB adult caught; however, hand collection enabled a number of adults and larvae to be collected. From the CRB specimens collected in the Philippines, an OrNV infected CRB adult was identified. Isolation of OrNV from infected CRB-G tissue will be undertaken to prepare inoculum for testing efficacy against CRB-G.

Recommendations:

- Isolate live OrNV from the infected CRB-G specimen and begin characterizing its potential efficacy as a biocontrol agent CRB-G.
- It is still important to continue searching for OrNV isolates (and other potential biocontrol agents) in regions known to harbor CRB-G populations, particularly where CRB caused palm damage is not reported as an issue.
- As the current CRB lure does not appear to be efficient at attracting CRB-G, new lures that can attractant CRB-G needs to be identified.

2. BACKGROUND

The coconut rhinoceros beetle (CRB),*Oryctes rhinoceros*, is a major pest of coconut palm, oil palm and other palm species. Palms are damaged when adult beetles bore into the crowns of palms to feed on sap. Tree mortality occurs when beetles destroy the growing tip (meristem). Immature beetles (grubs) do no damage. They feed on dead, decaying vegetation in breeding sites. Preferred breeding sites are dead, standing coconut stems, and piles of decaying vegetation such those left behind by typhoons or after replanting of oil palm plantations. If a CRB population is not suppressed, it is possible for a positive feedback cycle to initiate whereby adult beetles kill massive numbers of palms, thereby generating more food for even more grubs which turn into adults which kill even more palms. An outbreak following this scenario occurred in the Palau Islands during the late 1940s resulting in about 50% coconut palms being killed by CRB throughout the archipelago and 100% mortality on some of the smaller islands (Gressitt, 1953).

Following 40 years of no geographical range expansion, CRB is on the move in the Pacific. CRB has been detected for the first time at several Pacific Island locations including Saipan (2006), Guam (2007), Port Moresby, Papua New Guinea (2010), Oahu, Hawaii (2013), and Honiara, Solomon Islands (2015). Eradication of CRB is extremely difficult, having been achieved only once, on Niuatoputapu (Keppel) Island, an island with an area of only 16 km^2 belonging to the Kingdom of Tonga (Catley, 1969). Failing eradication, the usual response to CRB infestations during the second half of the 20th century was introduction of OrNV, the biological control agent of choice for this pest. OrNV attacks only *Oryctes rhinoceros* beetles, typically reducing CRB damage by up to 90% with population suppression lasting indefinitely (Bedford, 2013). OrNV is auto disseminated, meaning the pathogen is carried between breeding sites by CRB adults. Like many biocontrol agents, OrNV is density dependent, working best at high population densities. Highly pathogenic OrNV isolates suppress population growth to levels, which result in only minor damage.

Current invasions of Pacific Islands by CRB involve a new invasive biotype (CRB-G) that has escaped from biological control by OrNV (Marshall et al.). Discovery of *Oryctes rhinoceros* nudivirus in the 1960s enabled the successful management of populations in Pacific Island Countries. Augmentative release of OrNV continues to be an important mechanism for CRB management in both coconut and oil palm growing regions. For 40 years after adoption of this biocontrol strategy, no new outbreaks of CRB were reported from uninfested palm growing islands in the Pacific ensuring continuity of palm based village economies. However, the situation has recently changed. For the first time in 40 years, CRB invasion into completely new areas has been reported. Additionally, Pacific areas with established CRB populations (e.g. Palau) have reported increased severity and frequency of CRB damage. Common to all these areas is the high incidence of severe palm damage not seen since the introduction of OrNV. Initial attempts to introduce OrNV into the Guam CRB population (CRB-G) were unexpectedly unsuccessful, raising the possibility that the CRB-G population that invaded Guam become tolerant or resistant to the commonly applied OrNV isolates. Analysis of several CRB populations has demonstrated that, in addition to Guam, the CRB-G biotype is also found in Hawaii, Palau, and most recently in Port Moresby (PNG) and Honiara (Solomon Islands) during 2015.

Uncontrolled infestations of CRB may kill most palms within a few years, as it currently being observed. A worse case scenario can be triggered by a massive outbreak of adult CRB emerging from abundant breeding sites made by large amounts of decaying vegetation left in the wake of a typhoon. This is the situation in Guam, which as visited by Typhoon Dolphin in May, 2015. Very high feeding activity will kill mature coconut palms, leaving standing dead coconut trunks that are ideal breeding sites for subsequent generations of beetles. During a CRB outbreak, there will be an increased risk of further spread to uninfested islands throughout the Pacific. Palms are important on Pacific Islands for various reasons: as a cash crop for nuts, oil and lumber, as an ornamental tree appreciated by residents and tourists. On some of the smaller, more traditional islands the coconut palm is referred to as the tree of life. Here, this species is an essential natural resource

providing income, housing, food, oil, soap, clothing, mats, baskets, and other containers. The smaller, poorer Pacific islands will suffer the most if the spread of CRB-G cannot be controlled.

The objective of this project was to begin searching for new OrNV isolates that could be effective against the CRB-G haplotype. Native habitats provide a good opportunity to identify candidate OrNV isolates (and other potential biocontrol agents). OrNV is known to be widespread across the Philippines, where CRB is considered as native, and the CRB-G haplotype has been identified from here. Identification of an OrNV isolate with good efficacy against CRB-G would assist in preventing further mortality and damage to coconut palms on CRB-G infested Pacific islands (such as Guam), and slow or halt its spread into other areas of the Pacific region.

3. METHODS

3.1 Collection and DNA extraction of CRB tissue

CRB specimens were collected using both hand collection (larvae and adults) and 'DeFence Traps' (hanging Tekken fish gill netting) fitted with the CRB attractant, Oryctalure (www.chemtica.com). Gut tissue samples from live CRB specimens were dissected and used for DNA extraction because decaying tissue is often of limited value in terms of DNA quality. To ensure DNA quality was maintained, a 0.5 - 1 cm piece of the midgut tissue was submerged in monopropylene glycol (PPG), and stored at -20°C.

DNA was extracted from CRB tissue using a ZR Genomic DNA Tissue MiniPrep (Zymo Research) kits. DNA elution was carried out using 100 µl of elution buffer from the appropriate kit, and aliquots of eluted DNA samples were subsequently used for further analyses.

3.2 PCR-RFLP detection of CRB-G haplotype

The primers used to amplify a 523 base pair (bp) fragment of the CRB COI gene were C1-J-1718Oryctes (5'- GGAGGTTTCGGAAATTGACTTGTTCC -3') and C1-N-2191Oryctes (5'-CCAGGTAGAATTAAAATRTATACCTC -3') (Marshall et al.). Each 25 µl PCR reaction constituted 0.125 µl i-StarTaq DNA Polymerase (iNtRON Biotechnology), 2.5 µl 10x PCR buffer (iNtRON Biotechnology), 0.5 µl dNTP mixture (10 mM), 0.5 µl C1-J-1718Oryctes (10 µM), 0.5 µl C1-N-2191Oryctes (10 µM), 2 µl diluted (1 in 50) DNA template, and 18.75 µl water. PCR amplifications were performed in a C2100 (BioRad) thermocycler with a cycling profile of 35 cycles of 94°C denaturation (30 s), 50°C annealing (45 s), 72°C extension (1 min) with an initial denaturation of 3 min at 94°C and a final extension of 5 min at 72°C. A 5 µl aliquot of each PCR reaction was checked by agarose gel electrophoresis (1%, 0.5xTBE), stained with RedSafe (iNtRON Biotechnology) and fluorescence visualized over UV light. Photographs were recorded using an UVIdoc HD2 gel doc (UVItech). For RFLP analysis, successfully amplified COI PCR products (8 µl) were each combined with 0.2 µl Mse1 (10U/µl; New England BioLabs, NEB), 1 µl 10x NEB Buffer#4, 0.1 µl 100x NEB BSA and 10.7 µl water, and incubated at 37°C for 3 h. Digested samples (10 µl) were mixed with DNA loading dye, loaded onto on a 2% agarose gel in 0.5xTBE buffer. The gel was electrophoresed using 60 V for 1.5 h, stained with RedSafe and DNA fluorescence detected over UV light. Photographs were taken using an UVIdoc HD2 gel doc.

3.3 PCR detection of OrNV infection

The PCR protocol for detection of OrNV was based on that described in Richards et al. (Richards et al., 1999), and has been subsequently modified to distinguish infection from mere presence (e.g. incidental contact contamination). The primer pairs used to amplify a 945 base pair (bp) fragment of the OrNV genome were OrNV15a (5'-ATTACGTCGTAGAGGCAATC-3') and OrNV15b (5'-ATGATCGATTCGTCTATGG-3')(Richards et al., 1999). Each 25 µl PCR reaction contained 0.2 µl i-StarTaq DNA Polymerase (iNtRON Biotechnology), 2.5 µl 10x PCR buffer (iNtRON Biotechnology), 0.5 µl dNTP mixture (10 mM), 0.5 µl OrNV15a (10 µM), 0.5 µl OrNV15b (10 µM), 1 µl diluted DNA (paired reactions of 1 in 100 and 1 in 5000), and 19.8 µl water. PCR amplifications were performed in a C2100 (BioRad) thermocycler with a cycling profile of 35 cycles of 94°C denaturation (30 s), 50°C annealing (45 s), 72°C extension (1 min) with an initial denaturation of 3 min at 94°C and a final extension of 5 min at 72°C. An 8 µl aliquot of each PCR reaction was separated by agarose gel electrophoresis (1%, 0.5xTBE), stained with RedSafe (iNtRON Biotechnology) and fluorescence visualized over UV light. Photographs were recorded using an UVIdoc HD2 gel doc (UVItech). Detection of OrNV PCR product in the 1 to 5000 dilution was considered indicative of OrNV infection (unpublished data; validated in combination with gross visual inspection and histological analysis based on pathology description (Huger, 2005)).

3.3 Histopathology detection of OrNV infection

Histopathology studies of tissue displaying symptoms of OrNV infection. Dissected midgut tissue was fixed for in a 10% neutral buffered formalin solution. Standard paraffin embedding, serial sectioning and hematoxylin and eosin staining methods were carried out on samples by Gribbles Veterinary Services (Christchurch, New Zealand). Stained slides of the alimentary tract were examined under brightfield and differential interference contrast (DIC) optics using an Olympus BX50 upright microscope and photographed with an Olympus DP-72 digital camera.

4. RESULTS AND DISCUSSION

A total of 214 CRB larvae and adults were collected from within the Dumagate - Dauin region of Negros Island, Philippines. Tissue from 94 individuals was preserved for further analysis (see Appendix 1 for specimen details). Only one specimen (a hand caught adult female; Dug-42) displayed the symptoms of OrNV infection (thick, milky coloured gut; see Huger, 2005). From this pool of preserved tissue specimens, 16 representative samples (including Dug-42) were selected for molecular analysis to determine haplotype and OrNV infection status. A summary of the results is presented in Table 1. Amplification of the partial COI gene failed for specimens Dug-55, -56, and -57; therefore, the CRB-G haplotype status could not be determined from these specimens. As COI is present in all host cells, this meant that the OrNV infection status could also not be resolved, despite the observation of OrNV DNA in specimens Dug-55 and -. For the remaining 13 specimens, all were of the CRB-G haplotype, while only Dug-42 was positive for OrNV infection. Histopathology examination of Dug-42 gut tissue revealed diagnostic indicators of OrNV infection as described in Huger (Huger, 2005).

Since three separate methods indicated OrNV presence, we conclude that the Dug-42 tissue was infected with OrNV. Isolation of OrNV from Dug-42 tissue has been undertaken, and early results from testing this material on the OrNV permissive cell line DSIR-Ha-1179 have shown the excepted cytopathic effects (data not shown). If the early cell culture results are confirmed, the next step will be to conduct a pathogen challenge assay using the Dug-42 OrNV isolate against CRB-G.

Table 1: Summary of haplotype and OrNV infection status for CRB collected from Negros Island, Philippines.

Specimen	COI PCR	OrNV PCR	Virus present	Haplotype
Dug-1	+	-	No	CRB-G
Dug-2	+	-	No	CRB-G
Dug-16	+	-	No	CRB-G
Dug-17	+	-	No	CRB-G
Dug-18	+	-	No	CRB-G
Dug-19	+	-	No	CRB-G
Dug-42	+	+	Yes	CRB-G
Dug-43	+	-	No	CRB-G
Dug-44	+	-	No	CRB-G
Dug-45	+	-	No	CRB-G
Dug-55	-	+	Undetermined	Undetermined
Dug-56	-	+	Undetermined	Undetermined
Dug-57	-	-	Undetermined	Undetermined
Dug-62	+	-	No	CRB-G
Dug-63	+	-	No	CRB-G
Dug-69	+	-	No	CRB-G

5. RECOMMENDATIONS

- Isolate live OrNV from the infected CRB-G specimen and begin characterizing its potential efficacy as a biocontrol agent CRB-G.
- It is still important to continue searching for OrNV isolates (and other potential biocontrol agents) in regions known to harbor CRB-G populations, particularly where CRB caused palm damage is not reported as an issue.
- As the current CRB lure does not appear to be efficient at attracting CRB-G, new lures that can attractant CRB-G needs to be identified.

6. ACKNOWLEDGEMENTS

We thank Ian Iriarte for his assistance in collection of CRB specimens and dissection of tissue. We also thank Bonito for sharing his invaluable local knowledge and in helping with the collection of CRB specimens. This project work was funded through a USDA Farm Bill grant.

7. REFERENCES

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8. APPENDICES

Appendix 1: Details of CRB tissue specimens collected from Philippines in January 2017.

Date	Specimen ID	Site	Collection Method	Stage/Gender	Condition	PPG	FAA	Live
26/01/2017	Du1	Farm log2	Fallen log By hand	L3 -large	slow, reddish colour	1	1	1
26/01/2017	Du2	Farm log2	Fallen log By hand	L3 -large	slow, red all over (almost purple)	1	1	1
26/01/2017	Du3	Farm log2	Fallen log By hand	L3 -large	slow, reddish colour, stripped, a little purple	1	1	1
26/01/2017	Du4	Farm log2	Fallen log By hand	L3 -large	slow, reddish colour	1	1	1
26/01/2017	Du5	Farm log2	Fallen log By hand	L3 -small	slow, reddish colour	1	1	1
26/01/2017	Du6	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Du7	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Du8	Farm log2	Fallen log By hand	L2	normal	1	0	0
26/01/2017	Du9	Farm log2	Fallen log By hand	L2	normal	1	0	0
26/01/2017	Du10	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Du11	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Du12	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Du13	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0

26/01/2017	Du14	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Du15	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Du16	Lumber yard, Sibulan	Sawdust By hand	Male	vigorous, active, fatty, brown medium gut	1	1	1
26/01/2017	Du17	Lumber yard, Sibulan	Sawdust By hand	Male	reasonabley active, not fatty, thin light brown gut	1	1	1
26/01/2017	Du18	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Du19	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Du20	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Du21	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Du22	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Du23	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Du24	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Du25	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Du26	Lumber yard, Sibulan	Sawdust By hand	L3 -small	flacid	1	1	1
26/01/2017	Du27	Lumber yard, Sibulan	Sawdust By hand	L3 -small	flacid	1	1	1
26/01/2017	Du28	Lumber yard, Sibulan	Sawdust By hand	prepupa	flacid, very fatty, lots liquid	1	1	1
26/01/2017	Du29	Lumber yard, Sibulan	Sawdust By hand	prepupa	very fatty	1	1	1
26/01/2017	Du30	Lumber yard, Sibulan	Sawdust By hand	L3 -large	soft flacid posterior, firm anterior (white tissue)	1	1	1
26/01/2017	Du31	Lumber yard, Sibulan	Sawdust By hand	prepupa	very fatty	1	1	1
26/01/2017	Du32	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	1	1
26/01/2017	Du33	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	1	1
26/01/2017	Du34	Lumber yard, Sibulan	Sawdust By hand	L3 -small	normal	1	0	0
26/01/2017	Du35	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
26/01/2017	Du36	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
26/01/2017	Du37	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
26/01/2017	Du38	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0

26/01/2017	Du39	Lumber yard, Sibulan	Sawdust By hand	L3 -small	normal	1	0	0
26/01/2017	Du40	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
26/01/2017	Du41	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
28/01/2017	Du42	Grandma home	window by hand	Female	live, fatty, thick white gut	1	1	1
28/01/2017	Du43	Grandma home	window by hand	Male	live, thin brown gut (kept in same bottle as #42 for 2 days)	1	1	1
28/01/2017	Du44	Bascofadco coop, Bacong	By hand	Male	live, thin brown gut	1	1	1
28/01/2017	Du45	Bascofadco coop, Bacong	By hand	Male	live, thin brown gut	1	1	1
28/01/2017	Du46	Bascofadco coop, Bacong	By hand	Male	live, thin brown gut	1	1	1
30/01/2017	Du47	Ton home, Dauin	stumps/logs By hand	Male	live, fatty, thick brown gut	1	1	1
30/01/2017	Du48	Ton home, Dauin	stumps/logs By hand	Teneral Male	live, fatty, thick brown gut	1	1	1
30/01/2017	Du49	Ton home, Dauin	stumps/logs By hand	Male	live, thick brown gut	1	1	1
30/01/2017	Du50	Ton home, Dauin	stumps/logs By hand	Teneral Male	live, fatty, thick brown gut	1	0	1
30/01/2017	Du51	Ton home, Dauin	stumps/logs By hand	Male	live, thin brown gut	1	0	1
30/01/2017	Du52	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut	1	0	1
30/01/2017	Du53	Ton home, Dauin	stumps/logs By hand	Male	live, thick brown gut	1	0	1
30/01/2017	Du54	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut	1	0	1
30/01/2017	Du55	Ton home, Dauin	stumps/logs By hand	L3	found dead in breeding site log	1	0	1
30/01/2017	Du56	Ton home, Dauin	stumps/logs By hand	L2 (x6)	composite sample	1	0	1
30/01/2017	Du57	Ton home, Dauin	stumps/logs By hand	L3	found dead in log	1	0	1
28/01/2017	Du58	Bascofadco coop, Bacong	Fallen log by hand	L3	Healthy looking	1	0	0
28/01/2017	Du59	Bascofadco coop, Bacong	Fallen log by hand	L3	Healthy looking	1	0	0
28/01/2017	Du60	Bascofadco coop, Bacong	Fallen log by hand	L3	Healthy looking	1	0	0
28/01/2017	Du61	Bascofadco coop, Bacong	Fallen log by hand	L3	Healthy looking	1	0	0
1/02/2017	Du62	Aubrey's, Lunga	balcony light	Female	live, thin brown gut	1	1	1
1/02/2017	Du63	Aubrey's, Lunga	Pheromone trap	Male	live, thick brown gut	1	1	1

31/01/2017	Du64	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Du65	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Du66	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut, very fatty	1	1	1
31/01/2017	Du67	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Du68	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Du69	palm plantation, San Jose	stumps/logs By hand	Female	live, thin light brown gut ,	1	1	1
31/01/2017	Du70	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut, fatty	1	1	1
31/01/2017	Du71	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Du72	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Du73	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Du74	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Du75	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Du76	palm plantation, San Jose	stumps/logs By hand	Male	live, medium brown gut	1	1	1
31/01/2017	Du77	palm plantation, San Jose	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
31/01/2017	Du78	palm plantation, San Jose	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
31/01/2017	Du79	palm plantation, San Jose	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
31/01/2017	Du80	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Du81	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Du82	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Du83	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut , fatty	1	1	1
1/02/2017	Du84	Ton home, Dauin	stumps/logs By hand	Male	live, thin dark brown gut	1	1	1
1/02/2017	Du85	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut	1	1	1
1/02/2017	Du86	Ton home, Dauin	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
1/02/2017	Du87	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut	1	1	1

1/02/2017	Du88	Ton home, Dauin	stumps/logs By hand	Male	live, thin brown gut	1	1	1
1/02/2017	Du89	Ton home, Dauin	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
1/02/2017	Du90	Ton home, Dauin	stumps/logs By hand	Male	live, thin brown gut	1	1	1
1/02/2017	Du91	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut , fatty	1	1	1
1/02/2017	Du92	Ton home, Dauin	stumps/logs By hand	Male	live, thin brown gut	1	1	1
1/02/2017	Du93	Ton home, Dauin	stumps/logs By hand	Female	live, thin light brown gut	1	1	1
1/02/2017	Du94	Ton home, Dauin	stumps/logs By hand	pupa (male)	all white (messy inards)	1	1	1

4 Budget

Please see following page.

FY18 Budget

Cooperator Name: Aubrey Moore, University of Guam

ITEM	APHIS FUNDS
PERSONNEL:	
Post doc	$60,000
Graduate assistant	$35,000
Part time student technicians	$50,000
Subtotal	**$145,000**
FRINGE BENEFITS:	
for above personnel (27% * salary)	$39,150
Subtotal	**$39,150**
TRAVEL:	
Post doc relocation (incoming)	$6,000
Foreign exploration for an isolate of OrNV which is highly pathogenic for CRB-G	$30,000
Trip to AgResearch New Zealand for PI and Post doc training in OrNV propogation, diagnosis and bioassay technique.	$12,000
Subtotal	**$42,000**
EQUIPMENT	
Incubator	$17,500
Subtotal	**$17,500**
SUPPLIES	
Laboratory supplies	$12,450
Vehicle fuel and maintenance	$5,000
Computers and supplies	$5,000
Subtotal	**$22,450**
CONTRACTUAL	
AgResearch New Zealand (OrNV propogation and molecular diagnostics)	$60,000
Cell phone rental and service (2 units)	$2,000
Subtotal	**$62,000**
OTHER	
Salary reimbursement (Aubrey Moore; 10% FTE @$80k)	$8,000
Administrative fee (10% of total grant charged by Research Corporation of the University of Guam)	$37,344
Subtotal	**$45,344**
TOTAL DIRECT COSTS	$373,444
INDIRECT COSTS (not to exceed 15%)	$0
TOTAL	**$373,444**