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

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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.
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> 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