

## Farm Bill Work Plan - Fiscal Year 2018

Dates of Agreement: August 1, 2018 through July 31, 2019

<b>Cooperator:</b>	University of Guam		
<b>State:</b>	Guam		
<b>Project:</b>	Oryctes Nudivirus for Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle		
<b>Project funding source:</b>	Farm Bill Section 10007		
<b>Project Coordinator:</b>	Aubrey Moore		
<b>Agreement Number</b>	TBD		
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This Work Plan reflects a cooperative relationship between the University of Guam (the Cooperator) and the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ). It outlines the mission-related goals, objectives, and anticipated accomplishments as well as the approach for conducting a project entitled **Oryctes Nudivirus for Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle** and the related roles and responsibilities of the parties [e.g., APHIS role(s) and Cooperator role(s)] as negotiated.

### Project Personnel

The project coordinator, Dr. Aubrey Moore will be assisted by a post-doctoral insect pathologist, Dr. James Grasela, and a graduate student, Ian Iriarte. The graduate student will be hired using project funds from this Farm Bill project. However, the post-doc will be funded by a grant from the Department of Interior, Office of Island Affairs. If approved, funds from this Farm Bill grant will also be used to support continued collaboration with Drs. Sean Marshall and Trevor Jackson at AgResearch New Zealand. These insect pathologists are recognized world experts on microbial biocontrol of CRB.

### I) OBJECTIVES AND NEED FOR ASSISTANCE

*What relevant need or problem within the cooperator's mission area requires a solution in carrying out a public purpose of support or stimulation authorized by a law of the United States? How does the need or problem align with the mission area and strategic goals of APHIS?*

The population of coconut rhinoceros beetles (CRB) first detected on Guam in 2007 is genetically distinct from other populations of this major palm pest and is being referred to as the CRB-G biotype. CRB-G is resistant to *Oryctes nudivirus* (OrNV), 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 were 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, Rota Island, Commonwealth of the Northern Mariana Islands in 2017, Savo Island, Solomon Islands in 2017 and Malaita Island, Solomon Islands in 2017. It is significant that all of these new invasions involve CRB-G. Thus, CRB-G is a regional problem, 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 objective of this project is to stop an uncontrolled outbreak of coconut rhinoceros beetle biotype G which is rapidly killing palms on Guam. 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.

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. 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. continued 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.

## **II) RESULTS OR BENEFITS EXPECTED**

- Foreign exploration leading to discovery of a highly pathogenic strain of OrNV or other microbial biocontrol agent for CRB-G 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 introduction of the highly invasive CRB-G biotype to other Pacific islands and elsewhere.
- Continued development of image analysis methods during the island-wide survey 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.

### **III) APPROACH**

#### **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. After 4 cycles of this experiment, mortality in the treatment group continues to increase.

CRB-G tissue samples from the “Witch's Brew” bioassays have been sent to AgResearch New Zealand to test for OrNV. If the virus is detected, we will continue the bioassays in the hope of selecting highly pathogenic virus.

#### **2. 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. In 2016 USDA-APHIS invited international experts to discuss a Pacific-wide response to CRB-G at the International Congress of Entomology in Orlando. A second meeting for most members of this group is being planned as a symposium at the Society of Invertebrate Pathology Annual Meeting, which will be held in Australia during August, 2018.

#### **3. Foreign Exploration for an Effective Biocontrol Agent for CRB-G**

The primary objective of this project is to find an effective biocontrol agent, most likely an OrNV isolate which is highly pathogenic for the CRB-G biotype. Prior to the discovery of the virus-resistant CRB-G biotype on Guam in 2007, Pacific island CRB populations were controlled by OrNV isolates which provided self-sustaining population suppression resulting in very low levels of damage.

A first exploration for an OrNV isolate which can be used as a biocontrol agent for CRB-G occurred during January, 2017. Moore, Iriarte and Marshall did field work on Negros Island, Philippines, where CRB-G coexists with other CRB biotypes. CRB tissue samples were sent to Marshall's AgResearch New Zealand lab for CRB genotyping, detection of OrNV, and propagation/purification of OrNV. One of thirty CRB-G adults collected during the trip was infected with OrNV. Unfortunately, samples of this isolate proved not to be pathogenic when tested in bioassays using CRB-G on Guam.

A second foreign exploration is being planned for Taiwan. CRB-G tissue samples analyzed at the University of Hawaii indicated an 80% infection rate.

As soon as a likely biocontrol isolate is identified, it will be propagated *in vivo* and field released. A USDA-APHIS for import and release is already approved for this work.

#### **4. Coconut Palm Health Survey**

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.

##### **4.1 Survey Method**

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

We will use methodology developed during the FY17 Farm Bill project.

- A 360 degree camera affixed to the roof of a vehicle will be used to collect georeferenced, time lapse frames while driving along major roads on Guam.
- Initially, the health of each coconut palm within each frame will be evaluated by a human analyst who will quantify v-shaped cuts, bore holes, and dead standing trunks, etc.
- Human-classified coconut palm images will be used as a training set for building an automatic classifier using computer vision and machine learning.

If we are successful in building an automatic classifier for CRB damage. This inexpensive system could be deployed for monitoring trends in CRB damage in infested areas or for detecting new infestations in uninfested areas.

**A. The Cooperator will:**

**1. By function, what work is to be accomplished?**

The cooperator will perform activities outlined above.

**2. What is the quantitative projection of accomplishments to be achieved?**

**a. *By activity or function, what are the anticipated accomplishments by month, quarter, or other specified intervals?***

**This project is based on contingencies which preclude projection of an exact timeline.**

- The primary goal of this project is to find an effective biological control agent for CRB-G. This will most probably be an isolate of OrNV either discovered during foreign exploration or selected for within the “Witch's Brew” bioassays.
- If and when an effective biocontrol agent is found, it will be propagated and released as soon as possible. The PI has a USDA-APHIS permit which allows import and release of OrNV.
- A CRB damage survey will be established to measure changes to the health of coconut palms on Guam. This survey will use images from georeferenced time-lapse videos collected using a 360 degree camera system system developed during the previous Farm Bill grant project. Initially, tree health will be evaluated by a human operator viewing the images. Eventually, classified images will be used to build an automatic classifier using computer vision and machine learning software.

***b. What criteria will be used to evaluate the project? What are the anticipated results and successes?***

A semiannual coconut palm health survey will be initiated during the first month of the project so that a reduction in tree mortality and defoliation in response to biocontrol agent releases can be measured.

Success of the project will be evaluated by large reductions in tree mortality and defoliation by CRB-G.

**3. What numbers and types of personnel will be needed and what will they be doing?**

- Dr. Aubrey Moore, Dr. James Grasela, Ian Iriarte, and possibly Dr. Sean Marshall will participate in foreign exploration for effective biocontrol agents.
- Dr. James Grasela will perform bioassays on Guam to evaluate biocontrol candidates.
- The PI, his grad student, Ian Iriarte, will perform CRB damage surveys on Guam.

**4. What equipment will be needed to perform the work? Include major items of equipment with a value of \$5,000 or more.**

*a. What equipment will be provided by the cooperator?*

- The project will require a service vehicle. This vehicle will be provided by the University of Guam.
- Three incubators with temperature and humidity control have been purchased for rearing coconut rhinoceros beetles for bioassays and auto-dissemination.

*b. What equipment will be requested from APHIS on loan?*

None.

*c. What equipment will be purchased in whole or in part with APHIS funds?*

None.

*d. How will the equipment be used?*

The vehicle will be used to conduct island-wide surveys and the incubators will be used for rearing coconut rhinoceros beetles for bioassays.

*e. What is the proposed method of disposition of the equipment upon termination of the agreement/project?*

The equipment will remain with the cooperator.

**5. Identify information technology equipment, e.g., computers, and their ancillary components.**

- Android smart phones will be used for the island-wide coconut palm health survey. These will be used for GPS and datalogging in conjunction with Samsung Gear 360 degree cameras.

**6. What supplies will be needed to perform the work?**

**a. What supplies will be provided by the Cooperator?**

None.

**b. What supplies will be requested from APHIS (list supplies)?**

None.

**c. What supplies will be purchased in whole or in part with APHIS funds?**

Containers and rearing media for CRB to be used in laboratory bioassays.

**d. How will the supplies be used?**

For rearing CRB and bioassays.

**e. What is the proposed method of disposition of the supplies with a cumulative value over \$5,000 upon termination of the agreement/project?**

We do not anticipate having supplies with a cumulative value over \$5000 at the end of the project.

**7. What procurements will be made in support of the funded project and what is the method of procurement (e.g., lease, purchase)?**

Cooperator procurements shall be in accordance with OMB Circulars A-102 or A110, as applicable.

Two Android smart phones will be used for the island-wide coconut palm health survey. These will be leased under a service plan from a local telecommunications provider.

Contract with AgResearch New Zealand to provide additional diagnostics, DNA analysis, isolation and propagation of OrNV.

Salary reimbursement for Principal Investigator, 10% of FTE.

Administrative fee; 10% of total award, charged by the Research Corporation of the University of Guam.

**8. What are the travel needs for the project?**

- Local travel for coconut palm health surveys
- Foreign exploration for an effective biocontrol agent for CRB-G will be done in Taiwan where a population of the CRB-G biotype with an OrNV infection rate of 80% has been located.

**9. Reports:**

Submit all reports to the APHIS Authorized Department Officer's Designated Representative (ADODR). Reports include:

- a. Narrative accomplishment reports in the frequency and time frame specified in the Notice of Award, Article 4.
- b. Federal Financial Reports, SF-425 in the frequency and time frame specified in the Notice of Award, Article 4

**10. Are there any other contributing parties who will be working on the project?**

- The Guam Department of Agriculture may be recruited to assist with the island-wide coconut palm damage survey.
- Dr. Sean Marshall, AgResearch New Zealand will perform DNA analysis of CRB and OrNV and he will characterize and propagate isolates which are candidates for CRB-G biocontrol.

**B. APHIS Will:**

**1. Outline the Agency's (USDA APHIS PPQ) substantial involvement.**

- a. Include any significant Agency collaboration and participation
  - Provide input and oversight in the development and execution of the survey to ensure it meets national program goals and APHIS mission needs.
  - Provide funds to the cooperator to cover costs as outlined in the financial plan.
  - Provide additional guidance and/or technical assistance to the project coordinator, as requested.
    - Assist in clarifying survey methods and detection, as well as, identification resources, as needed.
  - Support the work and financial plan development by the cooperator.
- b. Project oversight and performance management
  - Notify the project coordinator of reporting deadlines.
  - Provide guidance in the compilation and submitting of reports and other administrative matters.
  - Maintain data spreadsheets showing due dates for reports, requests for allocation, forms submitted, tracked by the survey specialist.
  - Provide general oversight and quality assurance of the program.
- c. Provide the equipment requested by the cooperator in 4.b. & c.
- d. Provide the supplies requested by the cooperator in 6.b. & c.

**IV) GEOGRAPHIC LOCATION OF PROJECT**

- A. *Is the project statewide or in specific counties?*  
Statewide.
- B. *What type of terrain will be involved in the project?*  
Urban and natural areas.
- C. *Are there any unusual geographic features which may have an impact on the project?*  
None.

Laboratory work will be done at the University of Guam, Mangilao, Guam and at AgResearch, Christchurch, New Zealand. Foreign exploration for an effective biological control agent for CRB-G will be done in the Taiwan and possibly other locations. Coconut palm health surveys will be performed on Guam.



## V) DATA COLLECTION AND MAINTENANCE

All data and technical reports generated by this project will be immediately available on-line in an Open Science Framework project entitled “CRB-G Management” at <https://osf.io/5js9z/>.

## VI) TAXONOMIC SUPPORT

Taxonomic support is not required for this project.

- *If you do not need additional assistance taxonomic assistance, list the person(s) or institution who will perform the identification/diagnostics, and do not check B.*
- *If you need assistance, check B.*

A. Person(s) or Institution that will screen targets (Name & Contact Information) and level of screening/identification.

Aubrey Moore, Extension Entomologist  
University of Guam


OR

B. ☐ Request for taxonomic support.

## VII) SURVEY SUMMARY FORM

Not applicable because this project does not include a pest survey.

VIII) SIGNATURES

  
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Dean Lee S. Yudin, ROAR      Date 5/22/18

  
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~~Vernon Harrington, ADODR~~      Date 6/12/18  
Stuart Stein