Farm Bill Work Plan - Fiscal Year 2017

Cooperator:	**University of Guam**			
State:	Guam			
Project:	Oryctes Nudivirus for Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle			
Project funding source:	Farm Bill Section 10007			
Project Coordinator:	Aubrey Moore			
Agreement Number				
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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.

I) OBJECTIVES AND NEED FOR ASSISTANCE

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

Items 1, 2 and 3 are currently supported by my FY2016 Farm Bill (performance period: August 1, 2016 through July 30, 2017). Item 4, which establishes a semiannual coconut palm tree health survey, is a new activity.

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-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.

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. We will continue these witch's brew experiments and send beetle tissue samples to AgResearch New Zealand to test for OrNV.

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.

3. 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.

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.

- 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

4.2 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.

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?**

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

- The first 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.
- The PI will apply for renewal of an existing APHIS-PPQ permit to import and release OrNV so that candidate isolates can be released soon after discovery and completion of initial lab bioassays.

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

The semiannual coconut palm health survey will be initiated during the first 6 months 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?**

The PI and his grad student will perform biocontrol work and damage surveys on Guam. Dr. Sean Marshall, under a contract between UOG and Ag Research New Zealand, will DNA analysis of CRB and OrNV. He will also propagate candidate OrNV isolates in insect cell culture.

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

The only essential equipment to be used in this project is a service vehicle for the island-wide coconut palm health survey. This vehical will be provided by the University of Guam.

5. **Identify information technology equipment, e.g., computers, and their ancillary components.** All information technology supplies (e.g., small items of equipment, connectivity through air cards or high speed internet access, GPS units, radios for emergency operations etc.) should be specifically identified.

    - Android smart phones will be used for the island-wide coconut palm health survey.
    - Raspberry PI 3 computers will be used for digital image analysis.

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

    Supplies will include containers and rearing media for CRB to be used in laboratory bioassays.

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.

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

    - Foreign exploration for an effective biocontrol agent for CRB-G is contingent on results from the first expedition.
    - Progress on this project may require the PI and his grad student to visit Dr. Sean Marshall's lab in Christchurch, New Zealand to learn OrNV propagation technique. A trip by Dr. Sean Marshall to the University of Guam may be required to facilitate laboratory testing and field release of OrNV isolates.

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 <u>other</u> contributing parties who will be working on the project?

- The Guam Department of Agriculture will 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 isoltes 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

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 Philippines 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.

VII) SURVEY SUMMARY FORM

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

VIII) SIGNATURES

Dean Lee S. Yudin, ROAR	**Date**	**Vernon Harrington, ADODR**	**Date**

Detailed Financial Plan

Project Title: Oryctes Nudivirus for Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle

Cooperator Name: University of Guam

Agreement Number: TBD

Dates of the Agreement: TBD

ITEM	APHIS FUNDS	COOPERATOR FUNDS	TOTAL
PERSONNEL:			
Graduate assistant (Ian Iriarte)	$35,000		
Subtotal	**$35,000**	**$0**	**$35,000**
FRINGE BENEFITS:			
for above personnel (27% * salary)	$9,450		
Subtotal	**$9,450**	**$0**	**$9,450**
TRAVEL:			
Foreign exploration for an isolate of OrNV which is highly pathogenic for CRB-G	$40,000		
Subtotal	**$40,000**	**$0**	**$40,000**
EQUIPMENT:	$0		
Subtotal	**$0**	**$0**	**$0**
SUPPLIES:			
Laboratory supplies	$32,550		
Vehicle fuel and maintenance	$3,000		
Computer and supplies	$5,000		
Subtotal	**$40,550**	**$0**	**$40,550**
CONTRACTUAL:			
AgResearch New Zealand	$35,000		
Cell phone rental and service (2 units)	$2,000		
Subtotal	**$37,000**	**$0**	**$37,000**
OTHER:			
Salary reimbursement (Aubrey Moore(PI); 10% FTE @$80k)	$8,000		
Subtotal	**$8,000**	**$0**	**$8,000**
TOTAL DIRECT COSTS:	**$170,000**	**$0**	**$170,000**
INDIRECT COSTS (15% on Total):	**$30,000**	**$0**	**$30,000**
TOTAL:	**$200,000**	**$0**	**$200,000**
Cost Share Information	100%	0%	100%