**Applicant Information**
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**Basic Suggestion Information**
Suggestion Title: Biocontrol of Coconut Rhinoceros Beetle Biotype G
Which Goal Area (choose one):
Goal 6 – Enhance Mitigation and Rapid Response
Total Budget: $282,044

**Cooperator Information:**
If this suggestion is funded, will all the funding remain with the entity submitting this suggestion (excluding subcontracts)? (**Yes**/No)
If no, this is a group suggestion and each cooperator will enter into a separate agreement with APHIS. List the principal investigator, organization, state, and amount of funding each cooperator will receive, including yourself. The total of all budgets listed must equal the total budget for this suggestion.

**Abstract (500 words or less)**

A newly discovered biotype of *Oryctes rhinoceros*, coconut rhinoceros beetle (CRB-G), is rapidly killing coconuts and other palms on Guam. Uncontrolled outbreaks of CRB-G are also occurring in Papua New Guinea, Solomon Islands, and Palau. Eradication is being attempted on Oahu and Rota. Following a failed eradication attempt on Guam, CRB-G proved hard to control because this biotype is resistant to *Oryctes rhinoceros* nudivirus (OrNV), which was previously used as a very effective biological control agent for control of CRB outbreaks on Pacific Islands and elsewhere.

The primary objective of this proposed project is to stop the uncontrolled outbreak on Guam. Pacific-based entomologists working on the CRB-G problem agree that the most feasible solution is to find and release a new isolate of OrNV which is highly pathogenic to CRB-G. All previous OrNV releases on Pacific Islands prior to the invasion of Guam by CRB-G 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. Our plan is to search for effective OrNV isolates which are controlling Asian populations of CRB-G. To date, CRB-G has been detected in the Philippines, Indonesia, Thailand and Taiwan. Foreign exploration for an effective OrNV isolate began in 2017 with an expedition to Negros Island in the Philippines. OrNV infecting a single CRB-G adult was isolated, but we have not been unable to infect CRB-G adults collected on Guam with this isolate in laboratory bioassays. When an effective OrNV isolate is found, it will be propagated *in vivo* and released into the Guam CRB-G population under the terms of an existing USDA-APHIS import and release permit.

A secondary objective is to establish an island-wide monitoring system to track temporal and spatial changes in the extent of CRB damage to Guam's coconut palms. Damage symptoms such as v-shaped cuts to fronds, bore holes, and dead standing coconut palm stems are readily observed during roadside surveys. Survey data will be collected using a 360 degree digital video camera mounted on a truck. Initially, video images of coconut palm damage by CRB-G will be detected, classified and tagged by a technician. When a large number of images have been tagged, these will be used to train a fully automated CRB damage detection and monitoring system. This automated system may be useful as an early detection device for CRB.

**IT component** (including, but not limited to the development of databases, applications, or the purchase of hardware, software, services and related resources)**?** (Yes/**No**)

**Affiliation with Tribal Nations?** (Yes/**No**)

**Citrus Component?** (Yes/**No**)

**Offshore or foreign component?** (Yes/**No**)

**Technical Approach:**

## 1. Background

Coconut rhinoceros beetle (CRB), *Oryctes rhinoceros*, is a major pest of palms. Adults bore into crowns to feed on sap. A palm may be 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 types of organic matter including dead trees, green waste, saw dust, manure, compost, and even in bags of commercially packaged soil.

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 rhinoceros* 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. However, the preferred 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 Pacific island populations of this major palm pest and it is being referred to as the CRB-G biotype. 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, and Rota in 2017. 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.

The current, full-on CRB-G outbreak on Guam was triggered by Typhoon Dolphin which visited the island in May 2015. It was not a very strong typhoon by Guam standards,but it was the first one in more than a decade and it created a lot more damage than expected. Abundant piles of decaying vegetation became CRB breeding sites. Some of these new breeding sites were in villages were they could be managed. But most were inaccessible: in jungles and/or on military land. Within a few months, massive numbers of adults were emerging from breeding sites and severely attacking palms which started to die. Prior to Dolphin, we saw some heavily CRB-damaged palms,but very few dead ones. A self-sustaining positive feedback cycle began whereby large numbers of adult beetles attacked and killed large numbers of palms which became breeding sites which generated 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. If the Guam CRB-G infestation cannot be controlled, it is expected that most palms on the island will be killed and CRB-G will spread to other islands and beyond. If CRB-G invades smaller islands and atolls where coconut is *the tree of life*, a human tragedy will ensue. On larger islands, coconut and oil palm industries, tourism, and native ecosystems will be severely impacted.

Concerned Pacific-based entomologists are attempting to raise support for coordinated regional response to CRB-G. APHIS supported this effort by hosting a meeting at the International Congress of Entomology, Florida, 2016. Another meeting aimed at organizing a formal collaboration in response to CRB-G is planned as part of the 2018 International Congress of Invertebrate Pathology and Microbial Control and the 51st Annual Meeting of the Society for Invertebrate Pathology which will be held on the Gold Coast, Queensland, Australia from 12 - 16 August 2018.

Financial assistance for the proposed project will facilitate:

1. continued support of an international collaborative project with the goal of discovering an isolate of OrNV or other microbial biocontrol agent which can be used for long-term suppression of CRB-G populations
2. continued support for a graduate research assistant at the University of Guam
3. continued support for operation of an insect pathology laboratory at the University of Guam to evaluate candidate biocontrol agents discovered during foreign exploration
4. support for establishment and operation of island-wide CRB damage surveys on Guam

## 2. Project Staff

- PI: Aubrey Moore, PhD; Insect Ecologist
- James Grasela, PhD; Insect Pathologist; funded for 2 years by a grant from Dept. of Interior, Office of Island Affairs
- Roland Quitugua, MS; Plant Pathologist; Collaborator
- Ian Iriarte, BS; Graduate Assistant
- Sean Marshall, PhD; Insect Pathologist; Collaborator; Participation funded by a contract between the University of Guam and Ag Research

# 3. Objective 1: Find an OrNV Isolate which is Highly Effective for Long-term Suppression of CRB-G Populations

## 3.1 Regional Collaboration

Moore will continue to work with collaborators at AgResearch New Zealand, the Secretariat of the Pacific Community (SPC) and others to put together a regional collaboration with the objective of finding an effective biocontrol agent for CRB-G. A meeting aimed at organizing a formal collaboration in response to CRB-G is planned as part of the 2018 International Congress of Invertebrate Pathology and Microbial Control and the 51st Annual Meeting of the Society for Invertebrate Pathology which will be held on the Gold Coast, Queensland, Australia from 12 - 16 August 2018. Moore, Grasela, Quitugua and Iriarte will participate in this meeting.

## 3.2 Foreign Exploration for an Effective Biocontrol Agent for CRB-G

Foreign exploration in search of a microbial biocontrol agent for CRB-G is already underway.

During January, 2017, Moore, Iriarte and Marshall did field work on Negros Island,Philippines, were we had previously discovered a population of CRB-G. 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 was done by Dr. Sean Marshall in his lab at AgResearch New Zealand. All CRB samples collected during the trip were determined to be CRB-G biotype. Only one sample was infected with OrNV. This isolate was propagated in insect cell culture and samples were shipped to Guam for biossay. Field collected CRB-G field-collected adults were challenged by applying virus solution to mouth parts. No significant effects were observed in comparison to experimental controls (see technical report in the Prior Experience section).

A trip to Taiwan is being planned. Shizu Watanabe, University of Hawaii,reported an 82% OrNV infection rate in CRB-G collected from this island.

## 3.3 Establish Sustainable Biocontrol of CRB-G by Autodissemination

When bioassays indicate that an OrNV isolate is a potential biocontrol candidate, the virus will be propagated *in vivo* and released into the Guam CRB-G population by autodissemination. Otherwise, foreign exploration will continue until a pathogenic isolate is found.

On Guam, beetles for bioassays, *in vivo* propagation and 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-G. 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.

# 4. Objective 2: 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 monitoring system to track temporal and spatial changes in the extent of CRB damage to Guam's coconut palms. Damage symptoms such as v-shaped cuts to fronds, bore holes, and dead standing coconut palm stems are

readily observed during roadside surveys. Survey data will be collected using a 360 degree digital video camera mounted on a truck. Initially, video images of coconut palm damage by CRB-G will be detected, classified and tagged by a technician. When a large number of images have been tagged, these will be used to train a fully automated CRB damage detection and monitoring system. This automated system may be useful as an early detection device for CRB. Roadside surveys on Guam will be performed bimonthly.

# 5. Project Timeline

## Objective 1: CRB-G Biocontrol

**Month 1**

Foreign exploration trip to Taiwan to prospect for an isolate of OrNV which can be used as an effective biocontrol agent for CRB-G. (Shizu Watanabe, University of Hawaii, reports that a CRB-G population in Taiwan has an 82% OrNV infection rate.) Moore, Marshall, Grasela, and Iriarte will make this trip to Taiwan.

**Month 2-3**

Perform lab bioassays with samples collected during Taiwan trip.

**Month 4:**

If bioassays show that Taiwan isolates are viable candidates for CRB-G control, propagate *in vivo* and distribute samples to collaborators.

If Taiwan isolates are not biocontrol candidates, plan a second trip to a different CRB-G population (to be determined).

**Month 5:**

If Taiwan isolates are viable biocontrol agent candidates, continue *in vivo* propagation and begin field releases by autodissemination.

If Taiwan isolates are not biocontrol candidates, prospect for biocontrol candidates in a second CRB-G population.

**Month 6-7:**

If Taiwan isolates are viable biocontrol agent candidates, continue *in vivo* propagation and field releases by autodissemination.

If Taiwan isolates are not biocontrol candidates, perform lab bioassays of samples from the second CRB-G population.

**Month 8-9:**

If Taiwan isolates are viable biocontrol agent candidates, continue *in vivo* propagation and field releases by autodissemination.

If bioassays indicate that isolates from the second location are valid biocontrol candidates, share samples with collaborators, propagate OrNV *in vivo* and begin field releases by autodissemination.

**Month 10-11:**

If Taiwan isolates are viable biocontrol agent candidates, continue *in vivo* propagation and field releases by autodissemination.

If bioassays indicate that isolates from the second location are valid biocontrol candidates, continue to propagate OrNV *in vivo* and begin field releases by autodissemination.

**Month 12:**

Prepare final report.

## Objective 2: Establish a CRB Damage Monitoring System

Island-wide roadside surveys will be done bimonthly using images recorded by a 360 degree camera in time lapse mode. For the first 2 surveys, CRB damage symptoms will be tagged by human experts. Tagged images will be used to train an artificial neural network (ANN) detector/classifier. If the ANN detects and correctly classifies damage symptoms (>80% accuracy), subsequent surveys will be processed by the ANN, otherwise, further human-tagged images will be collected and added to the training set to see if this significantly increases accuracy of the ANN.

**Impact and Benefit:**

Describe the potential impact(s) and benefit(s) from the suggested project, include trade impacts and benefits, if appropriate.

- 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 of the highly invasive CRB-G biotype to other Pacific islands and elsewhere.
- Development of image analysis methods may lead to a small, inexpensive, automated CRB damage detector which could be mounted on a drone or a conventional vehicle. This device could be used for early detection or monitoring of CRB damage

**Prior Experience:**

This Farm Bill suggestion requests funding to build on progress during FY17 and FY18.

My latest Farm Bill progress report is attached as **FB17_report1.pdf**

Methods and results from the initial foreign exploration for an effective OrNV biocontrol agent in the Philippines is documented in:

Marshall, Sean and Aubrey Moore 2017. Molecular analysis of *Oryctes* rhinoceros collected from Philippines is attached as **AgR PH CRB PCR analysis Report_SM170615.pdf**

**Budget Plan (use template)**

See attached spreadsheet: **MooreFB19Budget.xlsx**

**Goal Validations**
Goal 6 – Enhance Mitigation and Rapid Response

Strategy 1: Does this suggestion develop, promote, and implement new control technologies, tools, and treatments for use in plant health emergencies and/or established pest programs? Examples for this Goal 6 strategy include quarantine treatments and biological control. (**Yes**/No)

Strategy 2: Does this suggestion enhance preparation for a plant pest emergency by improving the knowledge base, response options, and capabilities prior to the onset of a plant pest emergency? For example, the development and training of rapid response teams (ICS),

development of New Pest Response Guidelines and offshore approaches to developing management options for key invasive pests before they arrive. (Yes/**No**)

Strategy 3: Does this suggestion provide initial or short term funding to quickly implement programs that employ existing tools and initial responses protocols for the overarching goals of containment, control, or eradication immediately following the development of a plant health emergency? (Yes/**No**)

Strategy 4: Does this suggestion provide technical assistance prior to, during, and immediately following the development of a plant health emergency through the development of New Pest Response Guidelines (NPRG) for the potential introduction of exotic plant pests? (Yes/**No**)