Establishment of Self-sustaining Biological Control of Coconut Rhinoceros Beetle Biotype G in Micronesia

Aubrey Moore PhD
University of Guam College of Natural and Applied Sciences

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1. Project Description

This grant proposal is a request for funds totaling $249,804.50 to support a one-year project with a major goal of establishing effective, self-sustaining biological control of the coconut rhinoceros beetle (CRB) on Guam. *Oryctes rhinoceros*.

1.1. Background

The coconut rhinoceros beetle, *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 re-planting of oil palm plantations. If a CRB population is not suppressed, it is possible for a positive feed-back 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). A similar outbreak, initiated by Typhoon Dolphin (2015), is currently impacting Guam.

Following 40 years of no geographical range expansion, CRB is again "on the move" in the Pacific. CRB was recently detected for the first time at several Pacific Island locations including Saipan (2006), Guam (2007), Port Moresby, Papua New Guinea (2010), Oahu, Hawaii (2013), Honiara, Solomon Islands (2015), Rota, CNMI (2017), and Aguiguan, CNMI (2019).

Eradication of CRB has been attempted many times but is extremely difficult, having been achieved only once, on Niuatoputapu (formerly known as Keppel Island), a tiny island belonging to Tonga, with an area of only 16 km^2 (3% the area of Guam) (Catley 1969).

Failing eradication, the usual response to CRB infestations during the second half of the 20th century was introduction of *Oryctes* nudivirus (OrNV), the biological control agent of choice for this pest Jackson 2009 . OrNV attacks only CRB, typically reducing damage by up to 90% with population suppression lasting indefinitely (Bedford 2013). OrNV is auto-disseminated, meaning the pathogen is carried between feeding and breeding sites by CRB adults. Like many biocontrol agents, OrNV is density-dependent, working best at high population densities. After release, OrNV sustains itself within the CRB population, limiting damage to very low levels (See Appendix A.1: Self-sustaining biological control of CRB in Fiji using OrNV).

Current invasions of Pacific Islands by CRB involve a new invasive biotype that has escaped from biological control by OrNV. Discovery of OrNV nudivirus in the 1960s enabled the successful management of CRB populations in Pacific Island Countries (Huger 2005). Augmentative release of OrNV continues to be an important mechanism for CRB management in both coconut and oil palm growing regions. For about 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 by beetles not seen since the introduction of OrNV.

Initial attempts to introduce OrNV into the Guam CRB population were unexpectedly unsuccessful, raising the possibility that the population that invaded Guam is tolerant or resistant to the commonly applied OrNV isolates. Subsequent DNA analysis showed that the Guam population is genetically different from other populations in the region. On the basis of distinct genetics and tolerance to currently available OrNV isolates, the Guam population has been designated a new biotype, CRB-Guam (CRB-G) (Sean David Goldie Marshall et al. 2015; Sean D. G. Marshall et al. 2017).

DNA analysis from an ongoing survey has detected the CRB-G biotype in Guam, Hawaii, Palau, Port Moresby (PNG) and Honiara (Solomon Islands). Thus, current invasions in the Pacific involve the CRB-Guam biotype and it is expected that these populations are tolerant isolates of OrNV previously used as biocontrol agents. However, Recent work has identified OrNV isolates which are new biocontrol candidates for CRB-G. (See the **Recent progress** section below [1.1].)

Uncontrolled CRB-G outbreaks on islands may kill most palms within a few years and risk of accidental spread to other islands is high. A worse case scenario for a CRB infestation may 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, from large scale land clearing or large environmental destruction during a war. The current uncontrolled outbreak on Guam was initiated by Typhoon Dolphin which visited Guam in May, 2015. Massive amounts of decaying vegetation left in the wake of this storm provided abundant CRB breeding sites. Very high feeding activity by adults emerging from these breeding sites killed mature coconut palms, leaving standing dead coconut trunks which became ideal breeding sites for subsequent generations of beetles.

During a severe 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, coconut palm is referred to as *the tree of life*. On these islands, 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 spread of CRB-Guam cannot be controlled. If CRB-G infests islands and atolls where the coconut palm as the *tree of life*, islanders may have to migrate to larger population centers.

Recommended response to CRB-G invasions. Entomologists working on the CRB-G problem agree that the most feasible way to prevent massive palm mortality during outbreaks is establishment of biological control using an isolate of OrNV which is highly pathogenic to CRB-G (Jackson 2015; Vaqalo et al. 2015).

The concensus among Pacific-based entomologists is that the most feasable way to stop massive palm mortality during CRB-G outbreaks is to find a find and release a have met several times to plan a response to CRB-G invasions. In a special meeting on CRB-G at the XXVth International Congress of Entomology , the following strategic plan was suggested:

A coordinated regional project should be organized and adequately staffed and funded to accomplish 3 objectives:

1. Survey CRB populations throughout the Asian/Pacific region to delimit the geographical distribution of CRB-G and identify its centre of origin.

2. Survey CRB-G populations from the centre of origin to find isolate(s) of OrNV (or other pathogens) that are highly pathogenic for the CRB-G biotype.

3. Implement *in vivo* or *in vitro* propagation of selected OrNV isolates for auto-dissemination on islands infested with CRB-G.

The CRB-G problem is not limited to American-affiliated islands. Attempts to find financial support for a well-coordinated Pacific-wide response to this problem have failed. However, there is an *ad hoc* international community of entomologists, the *CRB-G Action Group*, which meets annually (Table 1.1).

Table 1: Meetings of the CRB-G Action Group

2015	Pacific Entomology Conference, Honolulu, HI, USA
2016	International Congress of Entomology, Orlando, USA
2017	Japanese Society for Insect Pathology, Tokyo, Japan
2018	Society for Invertebrate Pathology, Gold Coast, Australia
2019	XIX International Plant Protection Congress, Hyderabad, India
2020	(tentative): Pacific Plant Protection Organization, Guam

Recent progress. Recent work at the University of Guam, supported by grants from DOI-OIA and USDA-APHIS, has produced encouraging results (See Appendix C: *DOI-OIA Grant D17AP00107 Progress Report 4* for details):

- Laboratory tests indicate that OrNV from two sources can be considered as potential biocontrol agents CRB-G: OrNV isolate V23B maintained in insect tissue culture by AgResearch New Zealand and OrNV isolate UOGTW from bodies of CRB collected in Taiwan by the University of Guam CRB-G Biocontrol Project. Further laboratory testing of these virus samples is underway.

- PCR tests of recently collected CRB-G adults on Guam indicate presence of OrNV in this population. This virus could be from OrNV autodissemination earlier in the Guam CRB project or from fortuitous introduction.

1.2. Statement of Need

In addition to loss of coconut as ornamental plants and an emergency food supply, the uncontrolled CRB-G outbreak on Guam is a major environmental disaster rivaling that caused by the brown treesnake (BTS). BTS killed the birds in Guam's forests. CRB-G is now killing the trees. A 2002 US Forest Survey reported that the three most populous trees in Guam's forests were *Cycas micronesica*, *Cocos nucifera* and *Heterospathe elata* accounting for 16%, 12% and 11% of total trees with a stem diameter of five inches or greater (Moore 2018). These three species, 39% of the trees in Guam's forests, are being attacked by CRB-G[1]. If the Guam CRB-G outbreak is not brought under control, the island's forest health will continue to decline, accidental export of CRB-G to other islands in the American Pacific (in addition to Oahu and Rota) will be inevitable and cascading impacts from loss of forests will cause damage to other systems (erosion leading to reef fouling for example). Note that the source of the CRB-G invasion on Oahu and Rota is most probably Guam.

Despite the severity of the Guam CRB-G problem, the US federal government has provided relatively little support for response efforts. USDA-APHIS granted Plant Protection Act funding for CRB-G work in Hawaii and Guam for several years (Moore 2020). However, a grant proposal requesting $331,4904 for Guam from FY2020 PPA funding was unexpectedly rejected. A proposal requesting support ($3.5M) for a cooperative CRB biocontrol project to be performed by North Carolina State University, Colorado State University and the University of Guam has been submitted to DOD's Strategic Environmental Research and Development Program (SERDP). If granted, this SERDP project will begin in the middle of 2021. Thus, **work towards mitigating the CRB-G problem on Guam during FY2020 is currently unfunded**.

[1] Attack of *Cycas micronesica* by CRB-G adults is a recent discovery.

The immediate challenge is to procure bridge funding to retain the Guam Biological Control Project's insect pathologist, Dr. James Grasela, for an additional year so that we can begin propagation and field release of the two OrNV biological control candidates he has identified.

1.3. Goals and Objectives

Recent laboratory bioassays indicate that two recently tested OrNV isolates, V23B and UOGT are potential biocontrol candidates. There is also recent evidence indicating that there is already OrNV actively spreading within the Guam CRB-G population.

1.3.1. Objective 1: Survey to Determine Background OrNV Incidence

CRB adults collected from breeding sites and pheromone traps throughout Guam will by tested for presence of OrNV using PCR. Laboratory bioassays will be performed on OrNV isolated from these beetles to evaluate potential for biological control.

1.3.2. Objective 2: Establish Sustainable CRB-G Biocontrol by Autodissemination of OrNV

OrNV biocontrol candidates will be propagated *in vivo* using established methods (Huger 2005) and released into the Guam CRB-G population by autodissemination. Autodissemination involves infecting healthy CRB adults with OrNV. These infected beetles are then released at points dispersed throughout the island where they vector disease to conspecifics. A permit for field release of OrNV on Guam has already been obtained from USDA-APHIS. Field releases on CEMML on DOD land and by UOG on the rest of Guam. All released beetles will be marked by etching unique numbers on their elytra using a computer-controlled laser engraving system system already in use for this application at UOG.

Beetles for *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. Impact of virus releases will be monitored using pheromone traps and a novel roadside video analysis system (see Subsection 1.3.3). A subset of beetles captured in traps will be used to estimate the virus infection rate. Concurrent with virus releases, we will continue to screen OrNV isolates to find candidate biocontrol agents.

1.3.3. Objective 3: Establish Island-wide Monitoring Systems for CRB and Coconut Palm Health

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 to evaluate success of the proposed biocontrol project, or any other mitigation efforts. We intend to re-establish island-wide trapping and to establish a sustainable roadside video survey which uses artificial intelligence to detect CRB damage in dash-cam videos.

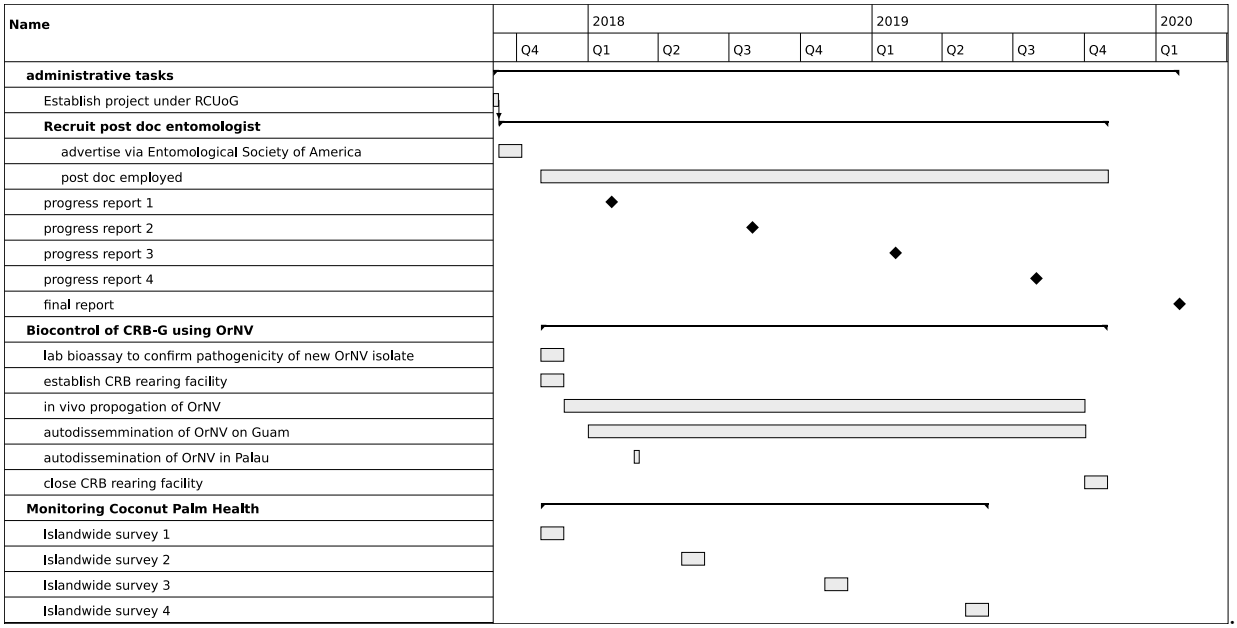
Pheromone Traps We plan to installed 150 CRB pheromone monitoring traps. These will be baited with oryctalure and serviced semimonthly. These traps catch approximately equal numbers of males and females which remain alive in the traps for several weeks. Collected beetles will be used for autodissemination of virus and a subsample will be used for virus detection. Traps will be deployed at least 3 months prior to initiation of autodissemination.

A web database already exists for Guam CRB trap data and it is available for use by this project (URL: **mysql.guaminsects.net**; database: **oryctes**; user: **readonlyguest**; password: **readonlypassword**; main tables: **trap** (2,265 records) and **trap_visit** (89,114 records)).

Roadside Surveys 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 on a smart-phone dash-cam app which georeferences each image. Initially, 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 an object detector. This work will result in a fully automated CRB damage detection and monitoring system which generates detection alerts and damage maps. This automated system will be useful as an early detection device for CRB. Roadside surveys on Guam will be performed bimonthly and the system will also be tested on Tinian, an island just north of Guam on which CRB has never been detected.

The envisioned system has already been successfully prototyped. A custom object detector for CRB damage has been trained using the TensorFlow implementation of the Faster R-CNN Deep Learning model (Moore, unpublished).

1.4. Timeline



1.5. Potential Benefits

- This project will directly benefit Guam and the Republic of Palau. Both of these jurisdictions are infested with CRB-G. Without implementation of effective biological control, it is likely that 50% or more coconut palms will be killed by CRB-G.

- This project will indirectly benefit all other islands in Micronesia. With very high populations of CRB-G in Gaum and the Republic of Palau, risk of accidental introduction to other islands is extremely high. CRB-G has already been intercepted twice on Saipan, Commonwealth of the Northern Mariana Islands. If CRB-G infests islands and atolls where the coconut palm as the *tree of life*, islanders may have to migrate to larger population centers.

- 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.

2. Budget

Item	Cost
Personnel	$207,170.00
Travel	$4,000.00
Supplies	$15,925.00
SUBTOTAL	**$227,095.00**
Administrative fee	$22,709.50
TOTAL	**$249,804.50**

Personnel includes salary and benefits for an insect pathologist (Dr. James Grasela, $83,200), a laboratory technician (Mr. Chris Cayanan, $60,360) and a field technician (vacant, $60,360). Benefits for these 3 positions are calculated at 30% * salary. The PI's salary compensation is calculated at 0.025 FTE * $130,000.

Travel includes airfare and other relocation expenses for Dr. Grasela who resides in Missouri.

Supplies includes laboratory and insect rearing supplies ($3,000) and fuel and maintenance for the project's field vehical ($4,000), purchase of 150 pheromone traps ($6,000), and purchase of 780 pheromone lures ($2,925).

Administrative fee is equal to 10% of the total grant award is charged by the Research Corporation of the University of Guam for services provided.

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A. Supporting Technical Data

A.1. Self-sustaining Biological Control of CRB in Fiji Using OrNV

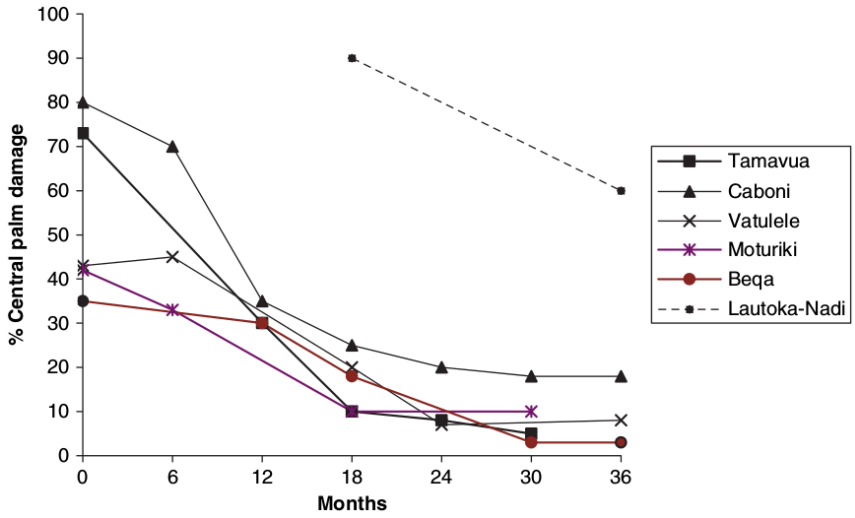

Figure 1: Reduction in coconut palm damage following release of *Oryctes rhinoceros* nudivirus in Fuji. (Jackson 2009)

Reduction in palm damage recorded over 36 months after release of *Oryctes virus* on five sites in the Fiji Islands from 1970 to 1972. No virus was released in the Lautoka area where damage remained high 18 months after the start of the program but natural incidence of disease was recorded in the area after 36 months coinciding with a decline in visible damage. Population suppression of CRB by OrNV in Fiji was still in effect 35 years after virus introduction (Bedford 2013).

A.2. Automated Monitoring of CRB Damage Using Roadside Video Surveys

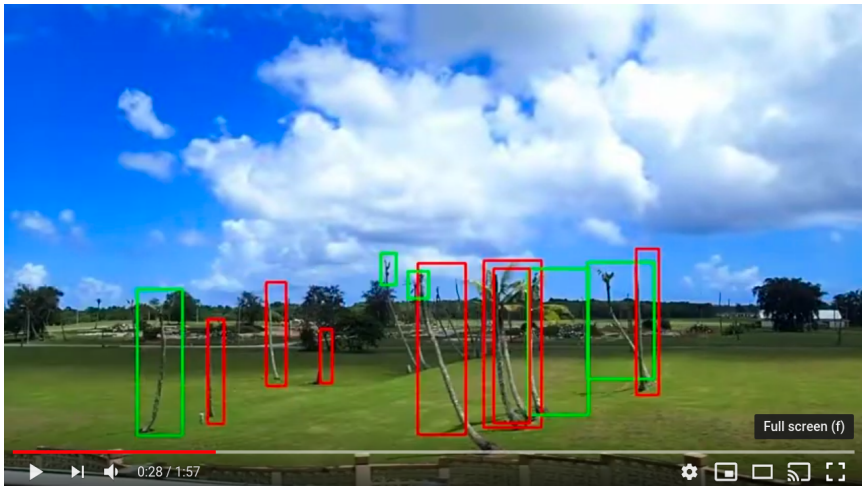

Figure 2: Training an Object Detector to Locate Coconut Palms Damaged or Killed by Coconut Rhinoceros Beetle. https://youtu.be/zzSorqcmt9U.

Result of a first attempt to train an object detector (Faster R-CNN) to locate coconut trees killed or damaged by coconut rhinoceros beetle in a video. Dead palms are in red boxes, damaged palms are in green boxes. Not perfect, but it does serve as a proof of concept.

B. USDA-APHIS 2020 Plant Protection Act Proposal (UNFUNDED)

Please see next page.

2020 USDA-APHIS Plant Protection Act Proposal

Coconut Rhinoceros Beetle Biological Control

Aubrey Moore
University of Guam College of Natural and Applied Sciences

Suggestion Information

Applicant Information

Name: Aubrey Moore Ph.D.
Email: aubreymoore@triton.uog.edu
Phone: 1-671-686-5664
Address (include zip code): 303 Campus Dr., UOG, Mangilao, Guam 96923
Cooperator Name: University of Guam College of Natural and Applied Sciences
Cooperator Type: Academia; 1890 Land Grant; State Government; Non-profit

Basic Suggestion Information

Suggestion Title: Coconut Rhinoceros Beetle Biological Control
Goal: Goal 6 – Enhance Mitigation and Rapid Response
Total Budget: $331,404

Additional Information:

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

Coconut rhinoceros beetle? (**Yes**/No)

Fruit fly? (Yes/**No**)

Spotted lantern fly? (Yes/**No**)

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

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

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

Benefits multiple states? (**Yes**/No)

Research or applied methods development? (**Yes**/No)

Cooperator Information:

If this suggestion is funded, will all the funding remain with the entity submitting this suggestion (excluding subcontracts)? (**Yes**/No)

Abstract (500 words or less, or approximately 3000 characters)

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 food source. 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. An eradication attempt using mass trapping and sanitation failed and the beetle spread to all parts of the island within a few years. Following this failure, *Oryctes rhinoceros* nudivirus (OrNV) and green muscardine fungus (GMF), *Metarhizium majus*, where introduced as biological control agents. GMF was successfully established as a classical biocontrol agent 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 Guam CRB population is genetically distinct from other Pacific island populations of pest and it is being referred to as the CRB-G biotype. While there where no range expansions of CRB for a quarter of a century (1980 to 2005), CRB is now on the move with 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. If outbreaks remain uncontrolled, CRB-G will continue to spread throughout the Pacific and beyond.

Pacific-based entomologists working on the CRB-G problem agree that the most feasible way to control CRB-G outbreaks on Pacific islands 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. Over the past 3 years we have worked with insect pathologists at AgResearch New Zealand and at the Tokyo University of Science and Technology to locate populations of CRB-G within the Asian-Pacific region and to isolate OrNV isolates from these populations. An insect pathologist, Dr. James Grasela, was recruited to work on CRB-G at the University of Guam funded with a 2 year grant from the US Dept. of the Interior. This proposal requests funding to continue collaboration with insect pathologists at AgResearch New Zealand and to support Dr. Grasela's post-doc position at UOG for a 3rd year.

Suggestion Body:

Purpose, Benefits and Accomplishments:

The primary objective of this proposed project is to stop the uncontrolled CRB-G 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.

Over the past 3 years we have worked with collaborators to obtain new isolates of OrNV infecting CRB-G populations in the Asian-Pacific region. Current laboratory bioassays indicate that at least one of these isolates may be an effective biological control agent for CRB-G. Selected OrNV isolates 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 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 an automated CRB damage detection and monitoring system using computer vision and deep learning. This automated system will be useful for monitoring results of biocontrol and other control activities. It may also be used as an early detection device for CRB.

Prior Experience:

Please see **Moore CRB Biocontrol Accomplish Report March 25 2019.pdf** (attached). This is the latest accomplishment report stored in ezFedGrants.

Budget Plan (use template)

Please see **Moore FY20 PPA budget.xlsx** (attached).

Technical Approach:

Objective 1: Establish Sustainable CRB-G Biocontrol by Autodissemination of OrNV

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. Autodissemination involves infecting healthy CRB adults with OrNV. These infected beetles are then released at points dispersed throughout the island where they vector disease to conspecifics.

Methods:

- On Guam, beetles for *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. These bioassays will require establishment and maintenance of CRB laboratory colonies.
- There will be an attempt to increase virulence by cycling isolates through several generations of beetles.

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.

Rather than re-establish a trapping survey, 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 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.

Methods:

- A protocol will be developed to perform roadside surveys of CRB damage. Damage will be recorded using videos recorded by a vehicle-mounted Olympus TG-5 camera. This camera records videos and GPS coordinates.
- Videos will be tagged using the open source Computer Vision Annotation Tool (CVAT).
- An object detector which locates and classifies CRB damage in video recordings will be trained using annotated videos from the previous step. We intend to use the TensorFlow implementation of the Faster R-CNN Deep Learning model. Training a CRB damage detector using deep learning requires use of a computer with specialized software (TensorFlow) and specialized hardware (a graphics processing unit (GPU)). Instead of purchasing a physical machine we will rent a virtual machine designed specifically for this application.
- Results from the trained object detector will be evaluated by comparison to human annotated videos.
- We will develop an automated processing system which takes roadside videos as input and generates CRB damage maps as output.

Milestones:

Objective 1: Establish Sustainable CRB-G Biocontrol by Autodissemination of OrNV

- **Month 1,2,3,4,5,6:** Introduce OrNV biological control candidates into the Guam CRB-G population by autodissemination.
- **Month 2:** Establish a breeding colony for CRB-G and CRB-S.
- **Month 3,4,5,6:** Perform laboratory bioassays to measure differences in responses to OrNV by CRB-G and CRB-S. In addition to mortality, we will measure differences in oviposition and flight capacity.
- **Month 7, 8:** Perform y-tube olfactometer trials to test for differences in attraction to orycatlure by CRB-G and CRB-S.

- **Month 7,8,9,10,11:** Perform surveys to measure spread of OrNV within the Guam CRB-G population.
- **Month 12:** Prepare final report.

Objective 2: Establish a Sustainable Coconut Palm Health Monitoring System

- **Months 1,3,5,7,9,11:** Island-wide roadside surveys will be done bimonthly using images recorded using an Olympus TG5 camera equipped with a GPS receiver mounted on a vehicle.
- **Month 1:** Annotate videos using the Computer Vision Annotation Tool (CVAT).
- **Month 2:** Use annotations to train an object detector for CRB damage using the Faster R-CNN model implemented in the TensorFlow object detection API. This work requires setting up a virtual machine with graphics processing unit (GPU).
- **Month 3:** Evaluate results from the trained object detector. If precision is insufficient, collect more annotated videos and add these to the training set.
- **Month 4:** Develop a software system which will take raw video GPS tracks as input, outputting CRB damage maps and statistics.
- **Month 12:** Prepare final report.

Goal Validations (Yes/No for each Strategy under the Goal you are submitting your suggestion for)

<u>Goal 6 – Enhance Mitigation and Rapid Response</u>
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**)

Aubrey Moore, University of Guam College of Natural and Applied Sciences

ITEM	APHIS FUNDS
PERSONNEL:	
insect pathologist post-doc (Dr. Jim Grasela) (1 FTE @ 60K)	$60,000
graduate Assistant (1 FTE @ $35K)	$35,000
insect rearing technicians (1 FTE @$30K; may be used to hire multiple student research assistants)	$30,000
survey technician (1 FTE @$30K; may be used to hire multiple student research assistants)	$30,000
Salary reimbursement for PI (Aubrey Moore; 10% FTE @ $110k)	$11,000
Subtotal	**$166,000**
FRINGE BENEFITS:	
27% * salary for all above, except salary reimbursement for Moore	$41,850
Subtotal	**$41,850**
TRAVEL:	
Travel to enable participation for PI and post-doc to meetwith collaborators and to participate in a national or international meeting.	$6,000
Subtotal	**$6,000**
EQUIPMENT	
Insect rearing chamber	$20,000
Eppendorf 5418 R Refrigerated Microcentrifuge	$6,699
Subtotal	**$26,699**
SUPPLIES	
Horizontal gel electrophoresis apparatus	$732
Gel electrophoresis power supply	$1,962
UVP Benchtop Transilluminator 110 v	$1,410
Molecular biology supplies (i.e. DNA extraction kits, virus isolation kits, PCR)	$2,463
Chemicals and reagents	$2,500
media for rearing beetles	$2,000
Vehicle fuel and maintenance	$5,000
Computers and computer supplies	$5,000
Cloud computing. Training a CRB damage detector using deep learning requires use of a computer with specialized software (TensorFlow) and specialized hardware (a graphics processing unit (GPU)). Instead of purchasing a physical machine we will rent a virtual machine designed specifically for this application from Amazon web services. We expect to run this machine for a total of 30 days. Estimated cost is $648 (30 d x 24 h x $0.90 per h)	$648
Subtotal	**$21,715**
CONTRACTUAL	
Contract with AgResearch New Zealand. Grant funding will be used to support an existing collaboration with Dr. Sean Marshall and Dr. Trevor Jackson who are recognized as global experts on biological control of CRB using OrNV. AgResearch New Zealand will provide molecular diagnostics for genotyping specimens of CRB and samples of OrNV. AgResearch maintains a collection of OrNV isolates in insect cell culture and has the facilities to mass produce virus *in vitro* once we have identified promising candidates for CRB-G biocontrol agents.	$35,000
Cell Phone Contract with Docomo Pacific. We rent a cell phone with unlimited data to be used as a dedicated device for CRB surveys. We commonly use the EpiCollect app for these surveys. This cell phone also doubles as a safety device for technicians working alone in remote locations.	$1,000
Subtotal	**$36,000**
TOTAL DIRECT COSTS	**$298,264**

INDIRECT COSTS (not to exceed 15%)	
INDIRECT COSTS Administrative fee (10% of total grant charged by Research Corporation of the University of Guam)	$33,140
TOTAL	**$331,404**

C. DOI-OIA Grant D17AP00107 Progress Report 4

Please see next page.

DOI-OIA Grant D17AP00107
Progress Report 4
Performance Period: March 1, 2019 - February 29, 2020

Coconut Rhinoceros Beetle Biological Control

Aubrey Moore, University of Guam

March 23, 2020

https://github.com/aubreymoore/doi-CRB-biocontrol-project/blob/master/report4/report4.pdf

Abstract

CRB Biological Control. The primary objective of this project is to find an isolate of *Oryctes rhinoceros* nudivirus (OrNV) which can be used as an effective biological control agent for the CRB-G biotype of coconut rhinoceros beetle (CRB).

Laboratory tests indicate that OrNV from two sources can be considered as potential biocontrol agents CRB-G: OrNV isolate V23B maintained in insect tissue culture by AgResearch New Zealand and OrNV in bodies of CRB collected in Taiwan for the current project. Further laboratory testing of these virus samples is underway.

PCR tests of recently collected CRB-G adults on Guam indicate presence of OrNV in this population. This virus could be from OrNV autodissemination early in the current project or from fortuitous introduction.

CRB Damage Survey. A secondary objective of this project is to develop a CRB damage monitoring system.

A digital image analysis system has been developed to detect and quantify V-shaped cuts to fronds and coconut palm mortality caused by CRB. The heart of this system is an object detector, trained by deep learning technology, which locates CRB damage symptoms on frames from georeferenced roadside video surveys. This object detector can be used to automate detection, quantification and to map changes in CRB damage over time and space and can also be used for early detection of CRB invasion.

A working prototype of the system has been built.

Regional Collaboration. Uncontrolled outbreaks of CRB-G is a major problem for Pacific islands. Outbreaks of this highly invasive biotype are damaging and killing palms in Guam, Rota, Hawaii, Palau, Papua New Guinea, and the Solomon Islands. Without effective control of these outbreaks, the problem will spread to other Pacific islands, resulting in a human tragedy when it reaches atolls were islanders still rely on coconut palm as the *tree of life*.

Project resources, time and effort were used to facilitate communication among an *ad hoc* collaboration of entomologists working on the CRB-G problem throughout the Pacific. Project staff participated in a symposium and planning meeting of the CRB-G Action Group at the XIX International Plant Protection Congress in November 2019.

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1 Background

The major goal of this project was to find an effective biological control agent for coconut rhinoceros beetle biotype G (CRB-G).

Prior to arrival of CRB-G on Guam during 2007, coconut rhinoceros beetle infestations of Pacific islands were readily dealt with by classical biological control using *Oryctes* nudivirus (OrNV), a pathogen specific to rhinoceros beetles. Following a lack of response to release of OrNV on Guam, research showed that the Guam CRB population is a genetically distinct virus-resistant biotype which has become known as CRB-G (Marshall et al. 2017). This biotype is highly invasive and is causing massive damage to coconut and oil palms after recent invasion of Papua New Guinea and the Solomon Islands. CRB-G has also invaded Oahu and Rota. Eradication attempts have been launched on these two islands.

Additional goals for this project are to establish a CRB damage survey to evaluate efficacy of biocontrol and other tactics, and to maintain and facilitate collaboration with other Pacific island entomologists working to find solutions for CRB-G management.

2 Staffing

Staff for this project currently comprises of the PI, a post-doc and a technician.

- Funding from the Department of Interior, Office of Island Affairs was used to hire an insect pathologist for a 2 year term. Dr. James Grasela was recruited and started work at UOG on June 24, 2018.
- Funding from this USDA-APHIS project was used to hire Mr. Chris Cayanan as a technician. Mr. Cayanan was hired during December, 2019, as a replacement for Mr. Ian Iriarte who resigned to accept another job.

3 Biological Control

3.1 Laboratory Bioassays of OrNV Isolates

Four isolates of OrNV were evaluated as candidate biological control agents for CRB-G in a series of laboratory bioassays. Virus sample preparations came from Dr. Sean Marshall's lab at AgResearch New Zealand were they are maintained in insect cell culture.

DUG42 Collected from Dumaguete, Negros Island, Philippines in 2017

MALB Collected from Malaysia, details not available.

PNG Collected from Rabaul, Papua New Guinea in 1988

V23B Collected from southern Luzon, Philippines in 1980

During laboratory bioassays, we dosed CRB-G adults with samples of the OrNV islolates, and observed mortality and changes in mass for one month. Each beetle was kept in isolation and individual records were stored in a laboratory information system developed for this application (Section 3.4.2).

Bioassay results, displayed in Table 1, indicate that one of the isolates, V23B, is pathogenic for CRB-G when doses are applied by placing droplets of virus suspension on mouthparts of adult beetles.

Table 1: *Oryctes rhinoceros* nudivirus (OrNV) bioassay results summary.

OrNV isolate	bioassay	method[1]	beetles	replicates	virus mortality (p)[2]	inactivated virus mortality (p)[3]
DUG42	DUG42 (Moore and J. J. Grasela 2019a)	injection	30	2	40% (0.65)	40% (0.65)
MALB	MALB (Moore and J. J. Grasela 2019b)	injection	30	2	50% (0.37)	0% (1.00)
	MALBperOS (Moore and J. J. Grasela 2019c)	per os	13	1	-60% (1.00)	20% (1.00)
PNG	PNG (Moore and J. J. Grasela 2019d)	injection	81	4	90% (0.00)	5% (1.00)
	PNGperOS (Moore and J. J. Grasela 2019e)	per os	21	1	0% (1.00)	0% (1.00)
V23B	V23B (Moore and J. J. Grasela 2019f)	injection	66	4	88% (0.00)	0% (1.00)
	V23BperOS (Moore and J. J. Grasela 2019h)	per os	32	2	80% (0.07)	20% (0.69)
	V23-large_bioassay (Moore and J. J. Grasela 2019g)	per os	53	1	42% (0.00)	-
	V23_perOSIN (Moore and J. J. Grasela 2019i)	per os	16	1	60% (0.06)	-

[1] Adult beetles were dosed either by direct injection of virus suspension into the haemocoel or by applying a droplet containing virus to mouthparts.
[2] Percent mortality in beetles treated with virus, adjusted for untreated control mortality; number in parentheses is the p-value resulting from a Fisher's exact test of significant difference between mortality of treated and untreated beetles.
[3] Percent mortality in beetles treated with heat inactivated virus, adjusted for untreated control mortality; number in parentheses is the p-value resulting from a Fisher's exact test of significant difference between mortality of treated and untreated beetles.

In a separate experiment, macerated guts from OrNV infected beetles from field collections of CRB in Taiwan were fed to CRB-G field-collected on Guam. PCR results indicated that the Taiwanese OrNV propagated in the Guam beetles (See Subsection 3.3).

We now have two isolates of OrNV which can be considered as candidate biocontrol agents for further testing: V23B and Taiwan.

3.2 Virus Transmission Experiment

This experiment was performed to determine if OrNV isolate V23B can be transmitted from a dosed CRB adult to an undosed CRB adult. If a virus is not contagious in the lab, it will probably have no potential as a biocontrol agent.

Unfortunately, the experiment failed due to very high mortality in the experimental control group and results are inconclusive. For details, see J. Grasela and Moore 2020. This experiment needs to be repeated using pathogen-free, laboratory-reared adults (See Section 3.4).

3.3 PCR Tests for OrNV Detection

Previously, our laboratory relied on outside collaboration for molecular testing to determine presence of OrNV in CRB tissues. We recently acquired access to equipment and supplies which allow us to use PCR (polymerase chain reaction) techniques for OrNV detection. We tested five different primer pairs for detection of OrNV in reference samples from AgResearch New Zealand and were successful with all five. Identity of DNA fragments was confirmed as OrNV using a commercial DNA sequencing service.

Details of our PCR technique and initial series of tests can be found in J. J. Grasela and Moore 2020a, J. J. Grasela and Moore 2020b and J. J. Grasela and Moore 2020c.

Results from PCR tests indicate:

- OrNV is present in the Guam adult CRB population: 18% (10 of 47) gut samples dissected from field collected CRB tested positive for OrNV.
- OrNV is present in the adult Taiwanese CRB population: 7% (5 of 67) gut samples dissected from field collected CRB tested positive for OrNV.
- OrNV from Taiwanese adult CRB propagates in Guam adult CRB: 12% (5 of 41) Guam CRB adults dosed with Taiwanese gut preparations tested positive.

3.4 CRB Rearing

Experimental beetles were field-collected on Guam as prepupae, pupae or adults from breeding sites or as adults from pheromone traps. Each beetle was given a serial number and was reared individually in a Mason jar stored in one of three environmental chambers set for 30 degrees Celsius, 80% relative humidity and 12 hour photoperiod. Each adult beetle was fed weekly with a slice of banana. Detailed information on the CRB rearing facility can be found in a document prepared in support of a USDA-APHIS permit to allow importation of live CRB for laboratory bioassays (Moore 2019a).

3.4.1 Urgent Need for a Pathogen-free CRB Laboratory Colony

To date, CRB used in bioassays and other laboratory experiments were field collected on Guam, rather than from reared in a laboratory colony. This was done because a very high population density of rhino beetles on Guam made field collection far more efficient in terms of time and resources than laboratory rearing. However, field collected beetles are often infected with pathogens, especially the fungus, *Anisopliae majus*, which was introduced on Guam as a classical biological control agent. Presence of these pathogens in experimental animals resulted in unpredictable and often high mortality rates in experimental control groups, leading to inconclusive results such as experienced with the recent virus transmission experiment (3.2).

Recent discovery of OrNV within the wild Guam rhino beetle population (3.3) now makes it mandatory to establish a sterile CRB-G laboratory colony to supply experimental animals.

3.4.2 Laboratory Information Management System

We developed an online database which we use as a laboratory information management system for maintaining individual records for beetles in our rearing program (Moore 2019b). This system was developed using the web2py python web framework and it is available on the web at `http://aubreymoore.pythonanywhere.com/rearing3`.

3.4.3 Acquisition of a Virus-Susceptible CRB Biotype for Comparative Bioassays

Since discovery of the CRB-G biotype on Guam (Marshall et al. 2017), we have been operating under the hypothesis that this biotype is significantly more tolerant (resistant) to pathogenic effects of OrNV isolates previously used as effective biocontrol agents for CRB invading Pacific Islands. It has also been hypothesized that CRB-G has different behavioral characteristics, such as a significantly reduced attraction to oryctalure. However, comparative laboratory bioassays have not been performed to test these hypotheses.

We applied for and have been granted a USDA-APHIS import permit for live CRB which will allow us to establish a laboratory colony of CRB from presumed non-virus-resistant populations (See (Moore 2019a) and (USDA-APHIS 2019)). We have designed custom shipping containers to facilitate secure transport of live CRB (Moore and Quitugua 2017).

We plan to import CRB from American Samoa with assistance from our collaborator, Dr. Mark Schmaedick, American Samoa Community College. Plans for project staff to visit American Samoa in December 2019 failed because of travel restrictions prompted by a measles outbreak and in March 2020 because of travel restrictions prompted by the corona virus pandemic.

4 CRB Damage Survey

The objective of this component of the project was to develop an automated system to evaluate CRB damage by image analysis of roadside video surveys. We completed a *proof of concept* trial in which deep learning algorithms were used to train an object detector which locates and counts dead and CRB-damaged coconut palms in video streams. Visual results are presented in a YouTube post (Moore 2019d) and technical details are available in an Open Science Framework Project (Moore 2019c).

5 Regional Collaboration

An informal collaboration, the *CRB-G Action Group*, has been formed among Pacific-based entomologists working on the CRB-G problem. Participants from Guam, Hawaii, Palau, Papua New Guinea, Solomon Islands, Fiji, Malaysia, Japan and New Zealand have met several times and future meetings are planned (Table 2). This is an *ad hoc* group which has been organized by Dr. Trevor Jackson and Sean Marshall of AgResearch New Zealand. AgResearch is recognized as a global center for expertise on biological control of CRB. AgResearch scientists have worked on CRB in the south Pacific for several decades and they maintain a library of OrNV isolates in cell culture. The New Zealand government has recently committed several million dollars to aid response to CRB-G in the south Pacific islands. Although individual institutions working to find a solution to the CRB-G problem on American-affiliated islands in the northern Pacific have secured funding from multiple, short-term grants, attempts to secure funding to support a sustainable well-coordinated regional project have been unsuccessful.

Table 2: Meetings of the CRB-G Action Group

2015 Pacific Entomology Conference, Honolulu, HI, USA
2016 International Congress of Entomology, Orlando, USA
2017 Japanese Society for Insect Pathology, Tokyo, Japan
2018 Society for Invertebrate Pathology, Gold Coast, Australia
2019 XIX International Plant Protection Congress, Hyderabad, India
2020 (tentative): Pacific Plant Protection Organization, Guam

5.1 Participation in Scientific Meetings

Moore and Grasela participated at the XIX International Plant Protection Congress in a symposium entitled *The challenge of coconut rhinoceros beetle, Oryctes rhinoceros, to palm production and prospects for control in a changing world*. Moore made an oral presentation at this meeting (Moore, J. J. Grasela, and Marshall 2019). They also participated in a CRB-G Action Group meeting with colleagues from throughout the Pacific and Asia.

5.2 Development of Online Resources

Project resources were used to build and maintain the following:

- CRB Bibliography https://github.com/aubreymoore/CRB-Bibliography
- Interactive CRB Invasion History Map https://aubreymoore.github.io/crbdist/mymap.html
- CRB Wiki Site https://guaminsects.net/CRBG
- CRB-G Facebook Site https://www.facebook.com/groups/crbg07

References

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D. Work Plan for CRB-G Biocontrol Project Funded by USDA-APHIS

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	17-8515-2058-CA			
Contact Information:	**Address:**	303 University Drive, Room 105 Agriculture and Life Sciences Building College of Natural & Applied Sciences University of Guam Mangilao, Guam 96923		
	Phone:	671-686-5664	**Fax:**	na
	Email Address:	aubreymoore@guam.net		

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 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. 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 introduction of the highly invasive CRB-Guam 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.

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. One additional trip to the Philippines by Moore, Marshall and Iriarte is planned. Moore and Iriarte will travel to Marshall's AgResearch lab in Christchurch for training in propagation of OrNV.

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 perform 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 project will require a service vehicle for the island-wide coconut palm health survey. This vehicle will be provided by the University of Guam.

Two incubators with temperature and humidity control will be purchased for rearing coconut rhinoceros beetles for bioassays and auto-dissemination. We will purchase the same units that are being used for CRB rearing at the

University of Hawaii so that we can share methodology. These Percival Scientific Biological Incubators Model I-41LLVL. These units are quoted at $17,500 each, including shipping to Guam.

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 equipped with HD cameras will be used for digital image analysis. Five units will be required for the project.
  - Computers: Raspberry atteryPi 3 (RPi3) Model B Quad-Core 1.2 GHz 1 GB RAM, On-board WiFi and Bluetooth Connectivity
  - Cameras: 5 megapixel native resolution sensor-capable of 2592 x 1944 pixel static images
  - Accessories: battery pack, realtime clock, case, microSD cards

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

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

6. **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.

   Two incubators with temperature and humidity control will be purchased for rearing coconut rhinoceros beetles for bioassays and auto-dissemination. We will purchase the same units that are being used for CRB rearing at the University of Hawaii so that we can share methodology. These Percival Scientific Biological Incubators Model I-41LLVL. These units are quoted at $17,500 each, including shipping to Guam.

7. **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 the Philippines where a population of the CRB-G biotype has been located.
- Progress on this project will require the PI and his grad student to visit Dr. Sean Marshall's lab in Christchurch, New Zealand to learn OrNV propagation technique.

8. **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.

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/.

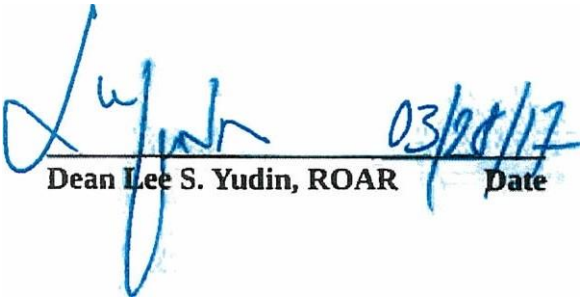
VI) TAXONOMIC SUPPORT

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



E. Extracts from the 22nd Micronesian Islands Forum Joint Communique

EDDIE BAZA CALVO
GOVERNOR OF GUAM
Chairman/Host

PETER CHRISTIAN
PRESIDENT OF THE FEDERATED STATES OF MICRONESIA
Member

TOMMY REMENGESAU, JR.
PRESIDENT OF THE REPUBLIC OF PALAU
Member

HILDA HEINE
PRESIDENT OF THE REPUBLIC OF THE MARSHALL ISLANDS
Member

RALPH D.L.G. TORRES
GOVERNOR OF THE COMMONWEALTH OF THE NORTHERN MARIANA ISLANDS
Member

JOHNSON ELIMO
GOVERNOR OF CHUUK
Member

LYNDON H. JACKSON
GOVERNOR OF KOSRAE
Member

MARCELO PETERSON
GOVERNOR OF POHNPEI
Member

JAMES YANGETMAI
LT. GOVERNOR OF YAP
Member

The Leaders called for the support of Micronesia Challenge Plus (MC+), which would fully integrate global and regional challenges, including Climate Change, Disaster Risk Management, and Invasive Species toward enhanced and holistic conservation measures and to move the 2020 commitments beyond 2020 and into the future. This will keep the MC in line with the revised and expanded UN Sustainable Development Goals.

2. Regional Invasive Species Council (RISC)

The Regional Invasive Species Council (RISC) reported on progress since the 21st MIF. RISC identified and discussed two major issues needing supporting action from the Leaders, and an additional two developments in the United States which needed to be brought to the attention of the Leaders. RISC also met with the MC SC to discuss ways that RISC can support the MC.

The first and most urgent issue is the need for regional coordination of invasive species activities in Micronesia, including implementation of the Regional Biosecurity Plan (RBP). RISC recommended the creation of a Regional Invasive Species Coordination Office to be staffed by a Regional Coordinator and to be housed in the Micronesia Center for a Sustainable Future (MCSF). The Regional Coordinator would be responsible to coordinate implementation of the RBP across the region, as well as to coordinate other activities and initiatives.

The second very urgent issue is the growing threat of the coconut rhinoceros beetle (CRB) to the region. CRB is devastating coconut trees in Guam, Palau, and other Pacific Islands. There is a high risk of their spread to other islands in Micronesia, and there is currently no effective control. A regional project is therefore urgently needed to develop an effective biological control for CRB.

RISC met with the MC SC to discuss ways that RISC can support the MC in their efforts to minimize the impacts of invasive species in terrestrial and marine conservation sites throughout the region as an integral component of effective conservation. Together, they identified the critical need to work together to establish baselines of invasive species in conservation sites. RISC will continue to assist the MC as they work to effectively conserve natural resources by protecting conservation sites from invasive species.

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GOVERNOR OF POHNPEI
Member

JAMES YANGETMAI
LT. GOVERNOR OF YAP
Member

- **Regional Coordination for Implementation of the Regional Biosecurity Plan (RBP)**

  The Leaders supported the establishment of a Regional Coordinator position in the to be established Regional Invasive Species Coordination Office housed in the MCSF. The Leaders committed themselves, through the MCSF, to provide initial funding support for the first two years to help ensure the effective implementation of the RBP.

- **US Presidential Executive Order on Promoting Agriculture and Rural Prosperity in America**

  The Leaders signed a joint letter to the Secretary of the US Department of Agriculture (USDA) welcoming the Executive Order signed by President Donald Trump on April 25, 2017, listing accomplishments of the Micronesian Region in the battle against invasive species and outlining needs for further progress. The Leaders look forward to working closely with the USDA and the Interagency Task Force to promote rural agricultural opportunity, food security and rural prosperity in the islands of Micronesia.

- **Regional biocontrol project for Coconut Rhinoceros Beetle (CRB)**

  In recognition of the urgent need for a Pacific-wide project to find an effective biological control agent for the CRB, the Leaders instructed RISC to seek financial support for such a project, to be conducted with partners at the University of Guam, the Secretariat of the Pacific Community, New Zealand, the USDA and others, as appropriate.

- **RISC/MC SC cooperation – capacity building for baseline surveys**

  The Leaders recognized the value of the collaboration between RISC and the MC SC and the progress they have made toward integrating invasive species prevention and management into effective conservation. The Leaders support the plan for RISC to work with the MC Measures Working Group to develop a capacity-building project to enable all jurisdictions to conduct baseline surveys for invasive plants, including development of a regional MC invasive species database for use in planning and decision-making. The Leaders expect that this will

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Member

22nd Micronesian Islands Forum

MAY 01, 2017 ~ MAY 03, 2017 I GUAM

MAY 0 2 2017

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Governor
U. S. Territory of Guam

TOMMY E. REMENGESAU, JR.
President
Republic of Palau

PETER M. CHRISTIAN
President
Federated States of Micronesia

HILDA C. HEINE, Ed. D
President
By Minister John M. Silk
Republic of the Marshall Islands

JOHNSON ELIMO
Governor
Chuuk State, FSM

RALPH D.L.G. TORRES
Governor
Commonwealth of the
Northern Mariana Islands

MARCELO K. PETERSON
Governor
Pohnpei State, FSM

for

LYNDON H. JACKSON
Governor
Kosrae State, FSM

JAMES YANGETMAI
Lt. Governor
Yap State, FSM

F. Letters of Support

F.1. Regional Invasive Species Council

Micronesia Regional Invasive Species Council

Tamdad Sulog
Chairman
Regional Invasive Species Council
P.O. Box 463
Colonia Yap FSM 96943

July 06, 2017

Nikolao Pula, Director
Office of Insular Affairs
U.S. Department of the Interior
1849 C Street, N.W. Mail-Stop 2429
Washington, D.C. 20240

Dear Director Pula,

As Chair of the Regional Invasive Species Council for the Western Pacific, I am pleased to submit a letter of support for Dr. Moore's grant proposal to support his work towards biological control of coconut rhinoceros beetle (CRB) on Guam and Palau.

Without a reducing the high populations of CRB on these islands, it is just a matter of time before this pest spreads within Micronesia. If it gets onto the smaller islands and atolls where coconut is the "tree of life" this will be a humanitarian tragedy.

RISC has been asked, via a communique from the recent Micronesian Forum, which is a meeting of all the Micronesian Governors, to help find a solution to the rhino beetle problem. Dr. Moore is offering a promising solution, and I urge your office to fund his proposal.

Yours sincerely,

Tamdad Sulog,
Chief, Yap Agriculture and Forestry

F.2. Republic of Palau

G. Administrative Forms

G.1. SF-424

OMB Number: 4040-0002
Expiration Date: 01/31/2019

APPLICATION FOR FEDERAL ASSISTANCE SF-424 - MANDATORY

1.a. Type of Submission:
- [X] Application
- [ ] Plan
- [ ] Funding Request
- [ ] Other

Other (specify):

1.b. Frequency:
- [X] Annual
- [ ] Quarterly
- [ ] Other

Other (specify):

1.c. Consolidated Application/Plan/Funding Request?
Yes [ ] No [X] Explanation

1.d. Version:
- [X] Initial
- [ ] Resubmission
- [ ] Revision
- [ ] Update

2. Date Received: 7/7/2017

STATE USE ONLY:

3. Applicant Identifier:

5. Date Received by State:

4a. Federal Entity Identifier:

6. State Application Identifier:

4b. Federal Award Identifier:

7. APPLICANT INFORMATION:

a. Legal Name: College of Natural and Applied Sciences, University of Guam

b. Employer/Taxpayer Identification Number (EIN/TIN): 98-0032933

c. Organizational DUNS: 779908151

d. Address:

Street1: 303 Campus Drive

Street2:

City: Mangilao

County / Parish:

State: GU: Guam

Province:

Country: USA: UNITED STATES

Zip / Postal Code: 96923

e. Organizational Unit:

Department Name:

Division Name:

f. Name and contact information of person to be contacted on matters involving this submission:

Prefix: Dr.

First Name: Aubrey

Middle Name:

Last Name: Moore

Suffix:

Title: Entomologist

Organizational Affiliation: College of Natural and Applied Sciences, University of Guam

Telephone Number: 1-671-686-5664

Fax Number:

Email: aubreymoore@guam.net

APPLICATION FOR FEDERAL ASSISTANCE SF-424 - MANDATORY

8a. TYPE OF APPLICANT:

H: Public/State Controlled Institution of Higher Education

Other (specify):

b. Additional Description:

9. Name of Federal Agency:

Department of the Interior

10. Catalog of Federal Domestic Assistance Number:

CFDA Title:

11. Descriptive Title of Applicant's Project:

Biological Control of Coconut Rhinoceros Beetle Biotype G in Micronesia

12. Areas Affected by Funding:

Guam, Republic of Palau

13. CONGRESSIONAL DISTRICTS OF:

a. Applicant: Guam

b. Program/Project:

Attach an additional list of Program/Project Congressional Districts if needed.

Add Attachment | Delete Attachment | View Attachment

14. FUNDING PERIOD:

a. Start Date: Sep 1, 2017

b. End Date: Oct 31, 2019

15. ESTIMATED FUNDING:

a. Federal ($): $176,553

b. Match ($): $0

16. IS SUBMISSION SUBJECT TO REVIEW BY STATE UNDER EXECUTIVE ORDER 12372 PROCESS?

- [ ] a. This submission was made available to the State under the Executive Order 12372 Process for review on:
- [ ] b. Program is subject to E.O. 12372 but has not been selected by State for review.
- [x] c. Program is not covered by E.O. 12372.

APPLICATION FOR FEDERAL ASSISTANCE SF-424 - MANDATORY

17. Is The Applicant Delinquent On Any Federal Debt?

Yes ☐ No ☒ Explanation

18. By signing this application, I certify (1) to the statements contained in the list of certifications and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances** and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 218, Section 1001)**

** I Agree ☐

** This list of certifications and assurances, or an internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

Authorized Representative:

Prefix: Dr.

First Name: Lee

Middle Name:

Last Name: Yudin

Suffix:

Title: Dean / Director

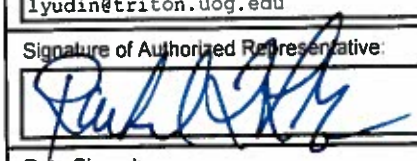
Organizational Affiliation: College of Natural and Applied Sciences, University of Guam

Telephone Number: (671) 735-2001

Fax Number:

Email: lyudin@triton.uog.edu

Signature of Authorized Representative:



Date Signed: July 6, 2017

Attach supporting documents as specified in agency instructions.

Add Attachments | Delete Attachments | View Attachments

G.2. SF-424A

BUDGET INFORMATION - Non-Construction Programs

OMB Number: 4040-0006
Expiration Date: 01/31/2019

SECTION A - BUDGET SUMMARY

Grant Program Function or Activity (a)	Catalog of Federal Domestic Assistance Number (b)	Estimated Unobligated Funds		New or Revised Budget		
		Federal (c)	Non-Federal (d)	Federal (e)	Non-Federal (f)	Total (g)
1. Biological control of coconut rhinoceros beetle	10.025	$	$	$ 176,553	$ 0	$ 176553
2.						
3.						
4.						
5. Totals		$	$	$	$	$

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Prescribed by OMB (Circular A -102) Page 1

SECTION B - BUDGET CATEGORIES

6. Object Class Categories	GRANT PROGRAM, FUNCTION OR ACTIVITY (1)	(2)	(3)	(4)	Total (5)
a. Personnel	$ 120000	$	$	$	$
b. Fringe Benefits	21600				
c. Travel	17298				
d. Equipment	0				
e. Supplies	0				
f. Contractual	0				
g. Construction	0				
h. Other	17655				
i. Total Direct Charges (sum of 6a-6h)					$
j. Indirect Charges	0				$
k. TOTALS (sum of 6i and 6j)	$	$	$	$	$
7. Program Income	$ 0	$	$	$	$

SECTION C - NON-FEDERAL RESOURCES

(a) Grant Program	(b) Applicant	(c) State	(d) Other Sources	(e)TOTALS
8. Biological control of coconut rhinoceros beetle	$ 0	$	$	$
9.				
10.				
11.				
12. TOTAL (sum of lines 8-11)	$	$	$	$

SECTION D - FORECASTED CASH NEEDS

	Total for 1st Year	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
13. Federal	$	$ 0	$ 0	$ 0	$ 35455
14. Non-Federal	$	0	0	0	0
15. TOTAL (sum of lines 13 and 14)	$	$	$	$	$

SECTION E - BUDGET ESTIMATES OF FEDERAL FUNDS NEEDED FOR BALANCE OF THE PROJECT

(a) Grant Program	FUTURE FUNDING PERIODS (YEARS) (b)First	(c) Second	(d) Third	(e) Fourth
16. 2018	$ 23998	$ 17700	$ 17700	$ 17700
17. 2019	17700	17700	17700	10900
18.				
19.				
20. TOTAL (sum of lines 16 - 19)	$	$	$	$

SECTION F - OTHER BUDGET INFORMATION

21. Direct Charges: 176553

22. Indirect Charges: 0

23. Remarks:

G.3. SF-424B

OMB Number: 4040-0007
Expiration Date: 01/31/2019

ASSURANCES - NON-CONSTRUCTION PROGRAMS

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE OFFICE OF MANAGEMENT AND BUDGET. SEND IT TO THE ADDRESS PROVIDED BY THE SPONSORING AGENCY.

NOTE: Certain of these assurances may not be applicable to your project or program. If you have questions, please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

As the duly authorized representative of the applicant, I certify that the applicant:

1. Has the legal authority to apply for Federal assistance and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project cost) to ensure proper planning, management and completion of the project described in this application.

2. Will give the awarding agency, the Comptroller General of the United States and, if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related to the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.

3. Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.

4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.

5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. §§4728-4763) relating to prescribed standards for merit systems for programs funded under one of the 19 statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).

6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C.§§1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation Act of 1973, as amended (29 U.S.C. §794), which prohibits discrimination on the basis of handicaps; (d) the Age Discrimination Act of 1975, as amended (42 U. S.C. §§6101-6107), which prohibits discrimination on the basis of age; (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-255), as amended, relating to nondiscrimination on the basis of drug abuse; (f) the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism; (g) §§523 and 527 of the Public Health Service Act of 1912 (42 U.S.C. §§290 dd-3 and 290 ee-3), as amended, relating to confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. §§3601 et seq.), as amended, relating to nondiscrimination in the sale, rental or financing of housing; (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and, (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.

7. Will comply, or has already complied, with the requirements of Titles II and III of the Uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally-assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.

8. Will comply, as applicable, with provisions of the Hatch Act (5 U.S.C. §§1501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

9. Will comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. §§276a to 276a-7), the Copeland Act (40 U.S.C. §276c and 18 U.S.C. §874), and the Contract Work Hours and Safety Standards Act (40 U.S.C. §§327-333), regarding labor standards for federally-assisted construction subagreements.

10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of the Flood Disaster Protection Act of 1973 (P.L. 93-234) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is $10,000 or more.

11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11988; (e) assurance of project consistency with the approved State management program developed under the Coastal Zone Management Act of 1972 (16 U.S.C. §§1451 et seq.); (f) conformity of Federal actions to State (Clean Air) Implementation Plans under Section 176(c) of the Clean Air Act of 1955, as amended (42 U.S.C. §§7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended (P.L. 93-523); and, (h) protection of endangered species under the Endangered Species Act of 1973, as amended (P.L. 93-205).

12. Will comply with the Wild and Scenic Rivers Act of 1968 (16 U.S.C. §§1271 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.

13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. §470), EO 11593 (identification and protection of historic properties), and the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. §§469a-1 et seq.).

14. Will comply with P.L. 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.

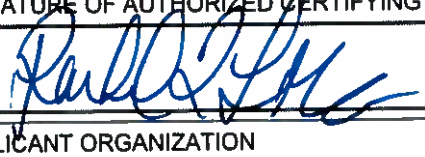
15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 89-544, as amended, 7 U.S.C. §§2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.

16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. §§4801 et seq.) which prohibits the use of lead-based paint in construction or rehabilitation of residence structures.

17. Will cause to be performed the required financial and compliance audits in accordance with the Single Audit Act Amendments of 1996 and OMB Circular No. A-133, "Audits of States, Local Governments, and Non-Profit Organizations."

18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations, and policies governing this program.

19. Will comply with the requirements of Section 106(g) of the Trafficking Victims Protection Act (TVPA) of 2000, as amended (22 U.S.C. 7104) which prohibits grant award recipients or a sub-recipient from (1) Engaging in severe forms of trafficking in persons during the period of time that the award is in effect (2) Procuring a commercial sex act during the period of time that the award is in effect or (3) Using forced labor in the performance of the award or subawards under the award.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL	TITLE
	Dean / Director
APPLICANT ORGANIZATION	DATE SUBMITTED