COOPERATIVE AGREEMENT WORK PLAN
Plant Protection and Quarantine, Science and Technology
and
University of Guam College of Agriculture and Life Sciences

Coconut Rhinoceros Beetle Biocontrol

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Contents

1 Introduction

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, Taiwan and Japan. 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 Guams 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 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.

2 Background

The primary purpose of this agreement is to stop an uncontrolled outbreak of CRB-G on Guam using biological control.

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 when 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 Guams 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 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 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 Guams 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 invaded by CRB-G, coconut and oil palm industries, tourism, and native ecosystems are being 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 was part of the 2018 International Congress of Invertebrate Pathology and Microbial Control and the 51st Annual Meeting of the Society for Invertebrate Pathology which was held on the Gold Coast, Queensland, Australia in August 2018. Future meetings specifically addressing international collaboration on CRB-G biocontrol include one at the International Association of Plant Protection Sciences meeting, Hyderabad, India 2019 and one tentatively being planned in association with the Pacific Plant Protection Organization on Guam during 2020.

Financial assistance for this project will support:

- 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

- continued support for operation of an insect pathology laboratory at the University of Guam to evaluate candidate biocontrol agents discovered during foreign exploration

- continued support for a graduate research assistant at the University of Guam

- support for establishment and operation of island-wide CRB damage surveys on Guam

Project Staff Funds from this grant are requested to support a graduate assistant and 3 part-time student workers. Collaboration with Dr. Sean Marshall, an insect pathologist at AgResearch New Zealand who is recognized as a world expert on biocontrol of CRB with OrNV, will be supported via a contract with his institution. Participation of other members of the project team is supported by other grants.

- 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

- Ian Iriarte, BS; Graduate Assistant

- Roland Quitugua, MS; Plant Pathologist; Collaborator

- Sean Marshall, PhD; Insect Pathologist; Collaborator; Participation funded by a contract between the University of Guam and AgResearch New Zealand

- 3 part-time student workers will assist with laboratory and field activities

3 Goals and Objectives

3.1 Objective 1: CRB Biocontrol

The primary objective is to find an OrNV isolate which is highly effective biological control agent for long-term suppression of CRB-G populations.

3.1.1 Regional Collaboration

Work will continue to work with colleagues at AgResearch New Zealand, the Secretariat of the Pacific Community (SPC), Tokyo University, the University of Hawaii and others to put together a regional collaboration with the objective of finding an effective biocontrol agent for CRB-G.

Methods

- Moore and Grasela will participate in the CRB-G biocontrol meeting at the IAPPS meeting in Hyderabad, India, November 2019.
- Moore will continue to maintain a web site to facilitate exchange of information on CRB-G biocontrol.

3.1.2 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 collected an isolate if OrNV from a CRB-G population in Negros Island, Philippines. Laboratory bioassays indicate that this isolate is not a good candidate for biocontrol.

We are currently performing laboratory bioassays to evaluate two novel isolates obtained from AgResearch New Zealand.

In addition we are attemping to islate OrNV from CRB adults collected in Taiwan. Dr. Shizu Watanabe, University of Hawaii,reported an 82% OrNV infection rate in CRB-G collected from this island.

Our next target population is CRB-G found on the southern islands of Japan. We plan to collaborate with Dr. Madoka Nakai, Tokyo University of Agriculture and Technology, to obtain CRB-G/OrNV specimens from these islands.

Methods

- Subsamples of CRB collected during foreign exploration will be shipped to AgResearch New Zealand to determine CRB biotype and to isolate OrNV
- OrNV isolates will be tested in the insect pathology lab at UOG using standard bioassay protocols.

3.1.3 Establish Laboratory Colonies of CRB-G and CRB-S

We will establish sustainable laboratory colonies of CRB-G and virus susceptible beetles (CRB-S) a source of healthy beetles for bioassays. Having these colonies will allow us to perform comparative bioassays to determine difference in tolerance to virus isolates and difference in behavior between the two biotypes.

Note: Establishment of a CRB-S colony is contingent on receiving a USDA-APHIS import permit to import live coconut rhinoceros beetles. I requested a permit on March 19, 2019 (Application number P526-190319-001) to replace a previous permit, P526P-11-01844, which I accidentally allowed to lapse into oblivion after only one shipment.

If we are allowed to import CRB-S, this will allow us to do comparative studies to:

- Measure difference in susceptibility to OrNV isolates. (Resistance of CRB-G to OrNV has not yet actually been proven by comparative bioassays.)
- Test for behavioral differences. (It has been hypothesized that the aggregation pheromone, oryctalure, is less attractive to CRB-G than CRB-S.)

Methods

- Larvae and adults will be reared individually in Mason jars enclosed by metal caps. Larvae will be fed a store-bought steer manure/soil blend on which we have reared CRB larvae for many years. Adults will be bedded in peat moss and fed banana slices weekly.

- Mason jars will be placed in envirommnental cabinets set at 30 dg. C, 80% RH and 12 h photoperiod.

- Detailed records for each individual beetle will be stored in an existing online laboratory information management system (LIMS). These data will be made available to USDA-APHIS.

3.1.4 Establish Sustainable CRB-G Biocontrol 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. Autodissemination involves infecting healthy CRB adults with OrNV. These beetles are then released at points dispersed throughout the island where they vector disease caused by OrNV 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.

- There will also be an attempt to increase virulence by cycling isolates through several generations of beetles.

3.2 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 Guams 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 will be recorded using a vehicle mounted Olympus TG-5 camera. This camera records 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 from Amazon Web Services.

- Results from the trained object detector will be evaluated using the human annotated videos.

- We will develop an automated processing system which takes roadside videos as input and generates CRB damage maps as output.

4 Milestones

4.1 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.

4.2 Objective 2: Establish a CRB Damage Monitoring System

Bimonthly 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 (¿80processed 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.

5 Methods

Methods are include with each objective listed in section(3).

6 Deliverables

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 Guams 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

7 Resources

7.1 Salary and Benefits

Graduate Assistant Ian Iriarte, BS; Graduate Assistant: **$35,000** per annum plus 27% benefits

Student Employees 3 part-time student employees to assist with laboratory and field activities 3*750h*$15/h=**$33,750** plus 27% benefits

Total Salaries **$68,750**

Total Benefits 0.27x(Total salaries) **$18,563**

Total Salaries and Benefits **$87,313**

7.2 Equipment

None.

7.3 Supplies

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).

Chemicals and reagents $2,500

Microinjector $2,747

pH meter $580

media for rearing beetles $2,656

Vehicle fuel and maintenance $5,000

Computers and computer supplies $5,000

Miscellaneous lab supplies $745

Total Supplies $19,876

7.4 Travel

International PI and Post Doc plan to participate in a special meeting on biocontrol of CRB-G at the International Association of Plant Protection Science meeting in Hyderabad, India, November 10-14, 2019. Estimated cost per person is **$5118** (Registration: $760; Airfare: $2048; Per diem: 7d@$330=$2310).

International PI and Post Doc plan a one week trip to Japan to work in collaboration with Dr. Madoka Nakai, Tokyo University of Agriculture and Technology, to collect isolates of OrNV from CRB-G populations in that country. January 15-22, 2020. Estimated cost per person is **$4099**. (Airfare: $550; Per diem: 7d@$507=$3549)

Total Travel 2x($5118+$4099)=**$17,334**

7.5 Contracts or Sub-Agreements

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**

Total for Contracts and Subagreements **$36,000**

7.6 Other

Salary reimbursement for PI (10% FTE @ $90k)=**$9,000**

Administrative fee (10% of total grant charged by Research Corporation of the University of Guam)=**$20,000**

Total Other **$29,000**

8 Budget

Salaries	\$68,750
Benefits	\$18,563
Equipment	\$0
Supplies	\$29,353
Travel	\$17,334
Contracts	\$37,000
Other	\$29,000
Total Direct Cost	\$200,000
Indirect Cost	\$0
Total Cost	\$200,000

9 Data

All laboratory data is stored in an online laboratory information system (LIMS) which we have custom built for this purpose. Results of bioassays are recorded in technical reports which are also accessible online.

The CRB damage survey component of the project is being developed as an open science project hosted on the Open Science Framework site.