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**)