

Coconut Rhinoceros Beetle Biological Control

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<https://github.com/aubreymoore/FY19-PPA-Report-1/raw/master/PPA19-report2.pdf>

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1 Objective 1: CRB Control

The primary objective is to find an OrNV isolate which is highly effective biological control agent for long-term suppression of CRB-G populations. As soon as laboratory studies indicate discovery of an OrNV isolate which is a potential biological control agent for CRB, we will multiply the virus in vivo and initiate field releases under the conditions of an existing USDA-APHIS permit.

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.

1.1.1 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.

1.1.2 Progress

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 of 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 attempting to isolate OrNV from CRB adults collected in Taiwan. This population was targeted because Dr. Shizu Watanabe, University of Hawaii, reported an 82

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.

1.2.1 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 collected during foreign exploration will be tested in the insect pathology lab at UOG using standard bioassay protocols.
- The PI and insect pathology post-doc will travel to Japan to collect CRB-G and OrNV,

1.2.2 Progress

1.3 Establish Lab Colonies of CRB-G and CRB-S

We will establish sustainable laboratory colonies of CRB-G and virus susceptible beetles (CRB-S) as a source of healthy beetles for bioassays.

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.)

1.3.1 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 environmental cabinets set at 30 deg. C, 80
- 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.

1.3.2 Progress

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.

2.0.1 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 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.

2.0.2 Progress

- CRB Action Group Webinar 1
- Presentation
- Rota webinar [\[1\]](#)
- Action Group Webinar 2

References

- [1] Aubrey Moore. “CRB Biology: Know Your Enemy”. Feb. 23, 2021. URL: <https://github.com/aubreymoore/CRB-CNMI/raw/main/CRB-Biology.pdf>.