Coconut Rhinoceros Beetle Biological Control

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https://github.com/aubreymoore/CRB-PPA19-Final/raw/main/PPA19-Final.pdf

Contents

Notes for the Reader

- Objectives and methods, as stated in the approved work plan [1] are presented in a frame at the start of each section.
- The University of Guam CRB biological control project is a long-term effort supported by multiple short-term grants. Some of the activities reported here have been partially funded by sources external to the FY19 APHIS-PPA CRB Biocontrol grant.
- Project staff:
  - Dr. Aubrey Moore, entomologist, PI
  - Dr. James Grasela, insect pathologist
  - Chris Cayanan, technician

1 Objective 1: CRB Biocontrol

1.1 Search for an Isolate of *Oryctes rhinoceros* Nudivirus for Biocontrol of CRB-G

The primary objective is to find an *Oryctes rhinoceros* nudivirus (OrNV) isolate which can be used as a 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.

1.1.1 Results

Bioassays done in our lab and concurrently in the Solomon Islands identified the V23B isolate of *Oryctes* nudivirus (OrNV) as a candidate biocontrol agent. This isolate was originally collected in the Philippines and it is maintained in cell culture at AgResearch New Zealand. Doses of the virus orally applied to adult beetles resulted in significant mortality and electron microscopy confirmed infection of gut cells [2].

Text within this box is copied *verbatim* from the approved work plan [1].

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 attempting to isolate OrNV from CRB adults collected in Taiwan. This population was targeted because 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 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.

1.2.1 Results

The project applied for and was granted USDA-APHIS permits to import *Oryctes rhinoceros* [3] and *Oryctes rhinoceros* nudivirus (OrNV) [4].

However, no progress was made on foreign exploration for OrNV isolates as potential biocontrol agents for CRB during the period covered by this report because of COVID-19 travel restrictions. See section 3.1 for details.

Text within this box is copied *verbatim* from the approved work plan [1].

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.)

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% 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.

1.3.1 Results

Background In recent years, we have used field collected beetles for lab experiments. Most came from pheromone traps, but some came from breeding sites. We used field collected beetles in preference to lab reared beetles because it was much easier to collect rather than rear. However, the quality of field collected beetles as test animals has proven highly variable. In several experiments, we observed high mortality in experimental control groups, resulting in failure of these experiments. We suspect that this mortality was due to pesticides and/or pathogens, especially *Metarhizium*, in their food.

We are developing a clean, well defined rearing protocol which will produce high quality CRB adults. We will to continue rearing insects individually in Mason jars. A record for each individual will be maintained in an existing laboratory management system (LMS) database. We intend to establish isofemale lineages starting with field collected gravid females. Care will be taken to maintain a pedigree for all progeny. When our rearing facility is in full production, DNA and live beetles from the UOG rearing facility will be made available to colleagues.

Larval Rearing Previously, we reared CRB individually in Mason jars. Beetles were reared from egg to pupa by feeding them commercial steer manure-soil mix purchased in large bags from a local hardware store. When adults emerged, steer manure was replaced with moist peat moss and were fed banana slices. Using a commercial steer manure product for CRB larval rearing is risky because cattle are often dosed with veterinary containing active ingredients such as ivermectin and abamectin which maintain insecticidal activity in manure. In addition, soil products are sometimes sprayed during production to control flies. We are currently transitioning from the commercial steer manure product to a natural diet for CRB grubs.

Standing dead, decaying coconut stems are a favored breeding site for CRB. Recent tests show that chipped material from standing dead coconut stems is also an excellent larval food source for CRB lab colonies [5]. The chipped material is stored in a chest freezer and autoclaved prior to use.

Each Mason jar is "seeded" with a surface-sterilized egg or neonate grub. Food is replaced as necessary during larval development.

Microbial symbionts picked up from the environment may be necessary for optimum CRB larval development. We are currently testing the idea of adding a small amount of frass when setting up Mason jars to see if this increases survival and weight gain of early instar grubs.

Adult Rearing Adults will be reared in the same Mason jars in which they pupated. Previously, we changed from coconut material to peat moss for adult rearing, but we are now trying to keep beetles in the same medium used for grubs. The only change is provision of a banana slice once per week for each adult.

Environmental Conditions Target conditions for rearing grubs and adults in Mason jars are 80 deg. F, 80% RH, and 12 h photoperiod.

The main rearing colony is kept in a converted 40 foot transmodal shipping container. Temperature is maintained by two air conditioners. Project funds were used to procure these air conditioners and also industrial shelving for the rearing colony.

Beetles being used in experiments are kept in 3 environmental chambers within a laboratory.

Establishment of a CRB-S Lab Colony UOG holds a USDA-APHIS permit to import live CRB to be reared in quarantine [3, 6] and we have built escape proof shipping containers for transporting live beetles [7]. Our original plan was to collect CRB-S from American Samoa. We had to cancel travel plans on two occasions, first because of a measles outbreak and second because of COVID. Our current plan is to source adult females from the Fiji. Unfortunately, this plan is still on hold pending relaxation of COVID travel restrictions.

Text within this box is copied *verbatim* from the approved work plan [1].

1.4 Regional Collaboration

Work will continue 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.

1.4.1 Monthly USDA-APHIS-PPQ Conference Calls

The PI regularly participated in monthly telephone conference calls hosted by Dr. Bill Wesela, APPHIS-PPQ National Policy Manager.

1.4.2 CRB Action Group Webinars

Table 1: Meetings of the CRB 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, December: CRB-G Action Group Webinar 1 [8]
2021, March: CRB Action Group Webinar 2 [9]
2021, November: CRB Action Group Webinar 3 [10]

The *CRB Action Group* is an ad hoc collaboration of Pacific-based entomologists and others working on the CRB-G problem throughout the Pacific. Participants from Guam, Hawaii, Palau, Papua New Guinea, Solomon Islands, Fiji, Malaysia, Japan, New Zealand, and Australia. The group met several times at international scientific meetings (Table 1).

During the COVID pandemic, the group has continued to meet via a Zoom webinars. Each webinar starts with island situation reports, followed by scientific presentations, and concluding with open discussion. A recording for each webinar is available online 1.

The PI served as an organizer for the Action group meetings, provided Zoom communications, and archived the meeting recordings.

1.4.3 Participation in Scientific Meetings

Moore and Grasela participated at the 2019 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 [11]. They also participated in a CRB-G Action Group meeting with colleagues from throughout the Pacific and Asia.

Moore and Jackson organized the 2020 CRB-G Action Group meeting as a Zoom webinar which occurred on December 9, 2020. Recordings of all sections of the webinar are available online [8]. A presentation on automated roadside surveys of CRB damage was presented at this meeting (recording and slide deck available at [8] and [12]).

Grasela made an oral presentation during the November 2021 CRB Action Group Webinar entitled *Preliminary efforts to establish a continuous CRB cell line.*

1.4.4 Rota CRB Eradication Project

Rota, an island in the Commonwealth of the Northern Mariana Islands about 35 miles north of Guam, was invaded by CRB with first detection in 2017. An eradication program initiated by USDA-APHIS is currently funded by the Department of the Interior - Office of Insular Affairs (DOI-OIA).

Moore collaborated with the Rota CRB Eradication Project on several occasions:

- In October 2020, a smart phone and associated equipment was sent to Rota-DLNR so that they could do an initial roadside video survey in support of their CRB control efforts. In addition to the equipment, a survey setup guide [15] and a setup video [16] were prepared and sent. See the Objective 2 section for details.

- On February 23 2021 DOI-OIA organized a webinar for the Rota CRB Eradication Project. Moore was invited to participate and made a presentation entitled *CRB Biology: Know Your Enemy* [13]. A recording of the webinar is available online [14].

1.4.5 Online Resources

Project staff maintain an online reference library [20], an online discussion group [19] and an online, interactive CRB invasion history map [18].

Text within this box is copied *verbatim* from the approved work plan [1].

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 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 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.

2.1 Results

We developed a highly automated roadside survey for CRB damage. High definition digital images are recorded along roadsides of all major routes at a rate of one per second by a smart phone attached to a vehicle [15], [16]. Back in the lab, a computer program developed using an artificial intelligence technique called *deep learning* examines every image, finds all coconut palms, measures CRB damage to each palm, and generates an interactive map which is published on the internet (Fig. 1).

Our survey is based on a standardized 5-point damage rating developed by Trevor Jackson at AgResearch New Zealand which is in use on several Pacific islands [12]. Automated damage rating is performed by a pair of object detectors developed under contract by OnePanel Inc. [21], [22], [23] which find all coconut palms within an image, score each one, and find v-shaped cuts. In addition to being used for surveillance, this automated system may also be useful for early detection of CRB.

A presentation on this new CRB survey methodology was made at the December 9 2020 meeting of the CRB-G Action Group conducted as a Zoom webinar [8], [12].

Guam surveys Five surveys have been completed on Guam:

1. October 2020 [24]
2. December 2020 [25]
3. March 2021 [26]
4. May 2021 [27]
5. August 2021 [28]

These surveys indicate that about 20% of Guam's roadside coconut palms show visible damage from coconut rhinoceros beetle (Fig. 1).

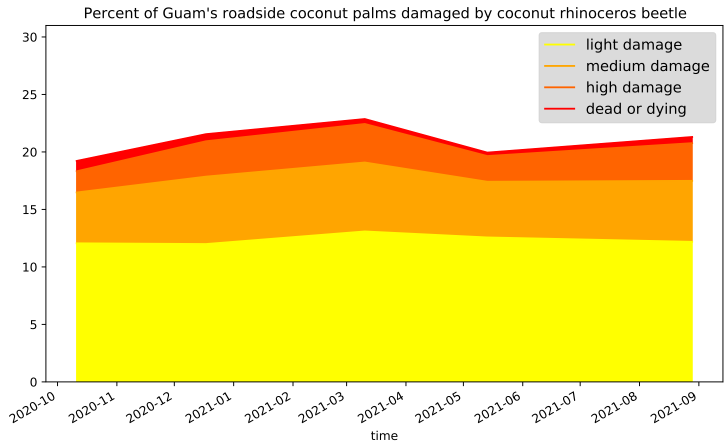

Figure 1: Coconut rhinoceros beetle damage ratings from automated roadside surveys.

The following four images were extracted from a draft of the University of Guam's Western Pacific Tropical Research Center impact report for 2020. They provide a nontechnical overview of the new automated roadside video survey for CRB damage and results from the first Guam survey in October, 2020.

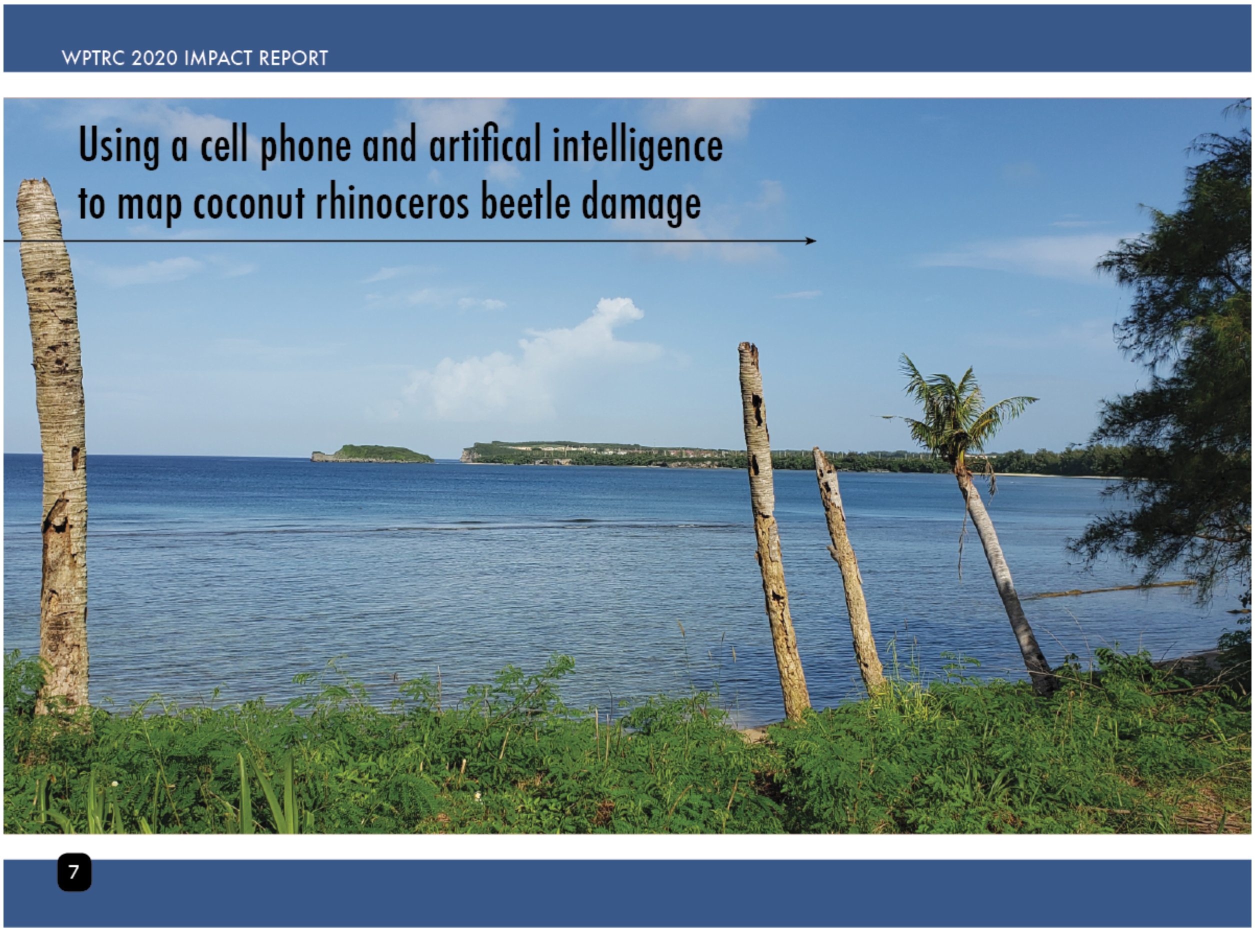

Figure 2: Feature article in the University of Guam's Western Pacific Tropical Research Center impact report for 2020.


WPTRC 2020 IMPACT REPORT

Everyone living on Guam has seen damage to coconut palms caused by coconut rhinoceros beetles (CRB). CRB has been on Guam since 2007, however, until recently, the number of palms being damaged and killed on Guam was unknown. Standardized surveys of CRB damage are needed to monitor changes over time and space, especially in response to control activities and for early detection of CRB in new geographic areas.

UOG entomologist Aubrey Moore has developed a highly automated method for routine island-wide monitoring of CRB damage using a cell phone and artificial intelligence (AI).

Methods for monitoring CRB damage have been developed. But these rely on direct observation or image analysis by human experts and are too time-consuming and expensive for routine monitoring over large areas.

Dr. Trevor Jackson, an entomologist working for AgResearch New Zealand, has developed a survey method based on a five-level scale for classifying CRB damage to individual coconut palms. Jackson's method is being used extensively on CRB-infested islands in the South Pacific. Moore decided to develop an island-wide roadside CRB damage survey for Guam based on an automated version of Jackson's method.

A smart phone is attached to a vehicle using a magnetic mount. As the car travels, the phone records videos that are analyzed by open-source software.

In the automated survey, a smart phone mounted on a car or truck records continuous videos while the vehicle is driven along all major roads on Guam. The smart phone uses a couple of free apps: OpenCamera records videos and GPSLogger records GPS coordinates.

Recent technical breakthroughs in AI have made it much easier to train computers to recognize objects in digital images. Moore collaborated with OnePanel Inc., an AI tech

Above: medium to severe CRB damage detected in the Royal Palms area of Dededo.

Below: each dot on the map represents a video frame in which one or more coconut palms was detected. The image at the top is a frame extract form a video with approximate at coordinates indicated by the camera icon.

Continue reading → 8

Figure 3: [Continued] Feature article in the University of Guam's Western Pacific Tropical Research Center impact report for 2020.

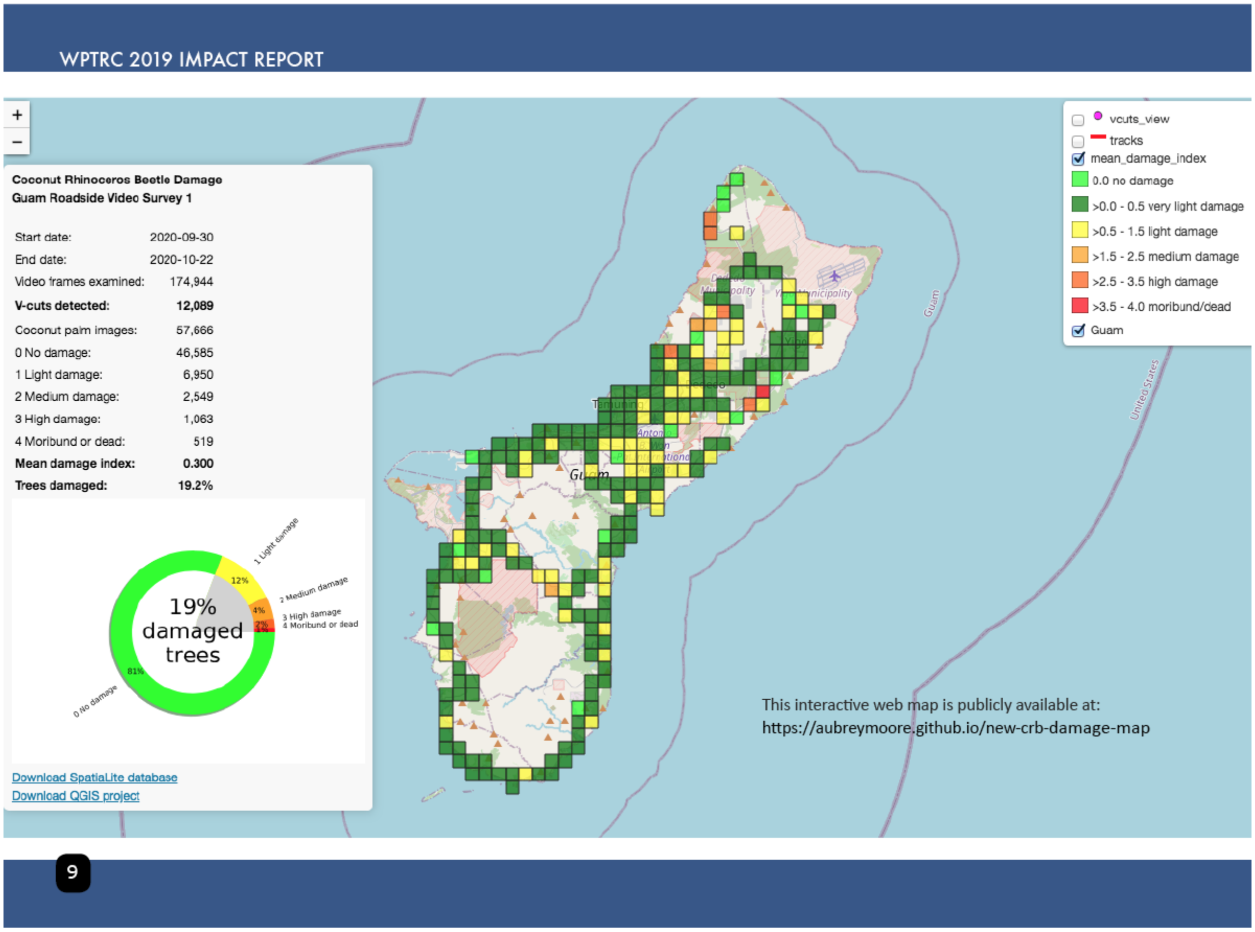

Figure 4: [Continued] Feature article in the University of Guam's Western Pacific Tropical Research Center impact report for 2020. This interactive web map is publicly available at: https://aubreymoore.github.io/new-crb-damage-map

company, to develop and train a couple of object detectors using a technique called “deep learning”.

The first object detector finds all images of coconut palms in the survey videos and classifies each one using Jackson’s damage scale. The second object detector locates and counts v-shaped cuts in the fronds of each coconut palm. Data extracted from the videos by the object detectors are saved in a SpatialLite database.

To visualize survey results, Moore uses Quantum GIS, to make a publicly available, interactive web map. There are links on the web map to download the survey database and QGIS map project for more detailed analysis.

The first operational island-wide survey on Guam, completed during October 2020, indicated that about 19% of Guam’s coconut palms show CRB damage symptoms. The Guam surveys will be conducted bimonthly. An island-wide roadside video survey is also being done on Rota for early detection of CRB damage.

There is interest in use of roadside video surveys for CRB damage elsewhere in the Pacific and Moore plans to evaluate drone imagery for use on islands without extensive roads. The Guam roadside video survey was designed to be adaptable by using only free

open-source software (FOSS) components. Custom-written software for the project as well videos, databases, and GIS projects from surveys will also be made available for download from public repositories hosted on GitHub.

It is interesting to note that this is not the first time that Moore has dabbled with AI. Thirty years ago he trained an artificial neural network to identify free-flying mosquitoes.

Funded by the US Department of the Interior-Office of Insular Affairs, US Forest Service, USDA-APHIS

Thanks to UOG entomology technician Christian Cayanan for doing the surveys.

Further reading:

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Figure 5: [Continued] Feature article in the University of Guam’s Western Pacific Tropical Research Center impact report for 2020.

Rota Survey Rota was invaded by CRB in 2017 and eradication efforts by Rota Department of Land and Natural Resources have successfully kept the population at a very low level, although the population has begun to spread to new areas of the island. In October 2020, a smart phone and associated equipment was sent to Rota-DLNR so that they could do an initial roadside video survey in support of their CRB control efforts. In addition to the equipment, a survey setup guide and [15] and a setup video [16] were prepared and sent.

The survey was performed by Mark Manglona, Rota-DLNR and the phone containing videos from the survey was returned to the University of Guam. Videos were analyzed using the workflow developed for the Guam surveys. The resulting web map contained many false positives for CRB damage, but there is one hit which shows a classic v-shaped cut probably caused by CRB. For convenience, data for this hit (images, date, location) were documented as an iNaturalist observation (Figure 6). If this v-shaped cut was caused by CRB, there will be a bore hole. Rota-DLNR located the damaged palm but did not find a bore hole. Therefor the damage was not caused by CRB.

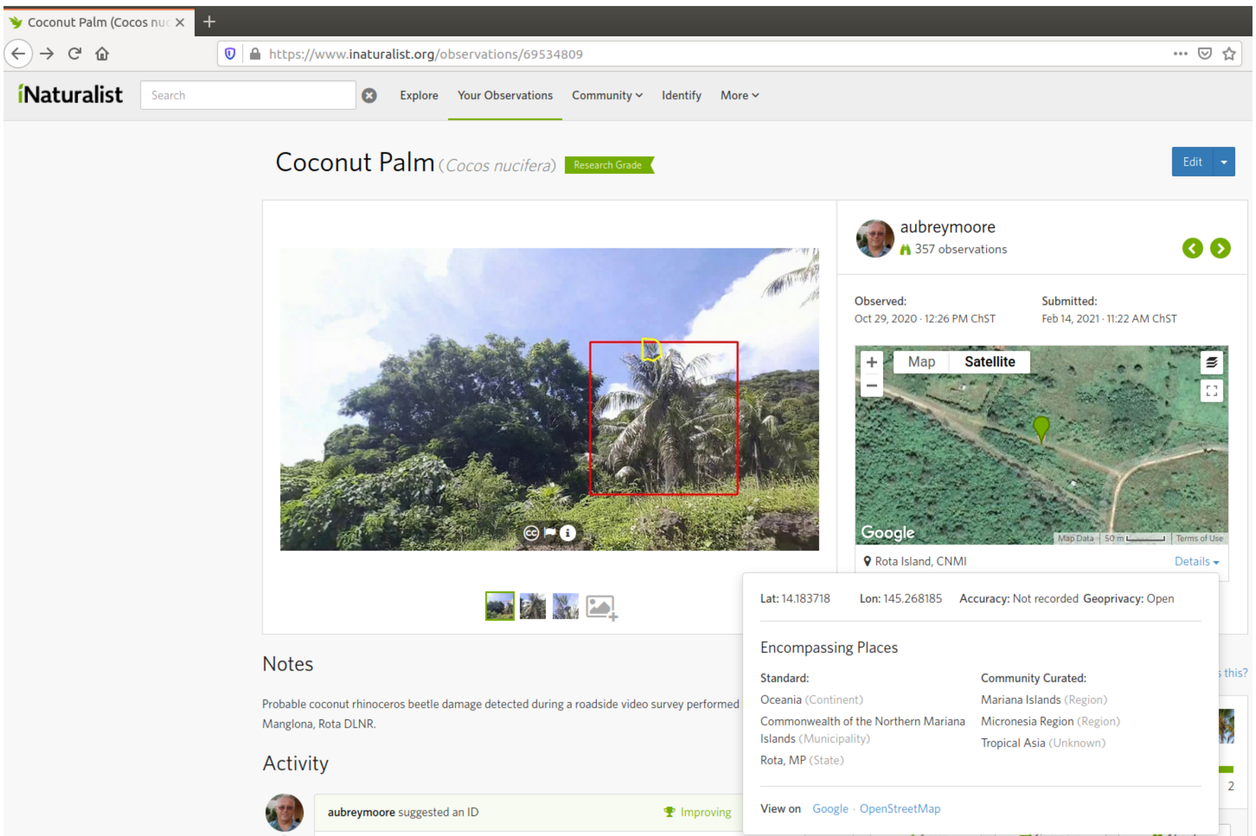

Figure 6: Screenshot of an iNaturalist observation documenting possible coconut rhinoceros beetle damage detected during a roadside video survey performed by Rota DLNR. https://www.inaturalist.org/observations/69534809.

3 Impediments to Progress in the Guam CRB Biocontrol Program

Significant progress was made on the Damage Survey and Regional Collaboration components of this grant. However, progress on the Biological Control component is lagging because of travel restrictions, stay at home orders, and a major technical problem.

3.1 Delays Caused by Travel Restrictions

The original work plan included foreign travel to collect novel OrNV isolates for evaluation as biocontrol agents and to collect virus susceptible CRB biotypes for comparative bioassays. Our first planned collecting trip was to visit American Samoa in December 2019. This trip was canceled at the last minute because of a measles outbreak. Our second attempt, in March 2020 was also canceled at the last minute, because of COVID-19 travel restrictions.

3.2 Delays Caused by COVID-19 Stay-at-Home Orders and University Closures

Progress was further delayed by Government of Guam stay at home orders. The University of Guam was officially closed from March 20 to May 10 2020 and again from August 16 2020 to January 15 2021.

3.3 Delays Caused by Detection of OrNV in CRB Collected on Guam

Our lab currently uses CRB-G adults collected from pheromone traps as test animals in bioassays evaluating OrNV isolates as biocontrol agents under the assumption that the Guam beetle population contains only the CRB-G biotype and is free from OrNV infection. In 2019 we gained the capacity to perform PCR in our lab began testing these assumptions. PCR results indicated that field-collected beetles were all CRB-G, but 18% of these tested positive for OrNV.

Based on these results, the PI decided to suspend bioassays until we had conclusive evidence of OrNV infection in the Guam CRB-G population. An experimental plan [29] was developed and executed. One hundred beetles were collected from each of two trapping sites (Leo Palace Resort in Southern Guam and the UOG Ag. Expt. Stn. in northern Guam). Gut samples were obtained from these beetles and tested using PCR in our lab and also in Sean Marshall's lab at AgResearch New Zealand. In PCR results from both labs all beetles tested positive for CRB-G biotype and negative for OrNV infection [30]. In addition, histological examination of gut samples did not detect OrNV inffection in any CRB collected on Guam [17].

We suspect that previous OrNV positive tests were the result of lab contamination (not false positives). During the hiatus in OrNV bioassays, we re-examined our bioassay methodology and have decided to make major changes before moving forward:

Re-establishment of a CRB rearing program. High variance among results from bioassay replicates and high mortality rates are a serious impediment to progress in finding an OrNV isolate which can be used as an effective biocontrol agent for CRB-G. Beetles collected from pheromone traps are not ideal test insects because they vary in age and many are infected with *Metarhizium majus*. We intend to re-establish the Guam CRB rearing program to supply healthy, standardized test insects. Insects will be reared individually in Mason jars and a detailed record will be maintained for each individual. Larvae will be fed a diet of heat-sterilized CRB breeding site material from dead standing coconut trunks. The CRB-G lab colony will be initiated with surface-sterilized eggs and isofemale lineages will be maintained. Because the life cycle of the coconut rhinoceros beetle is about 9 months, there will be a lag time of about one year before the rearing program is fully operational and bioassays can resume.

Measurement of sublethal impacts of OrNV infection. The literature indicates that reduction in damage to coconut palms after release of OrNV may be the result of sublethal impacts on the population rather than mortality. These impacts may include reduction in fecundity, feeding, and flight. Bioassays which measure only mortality may reject promising biocontrol candidates. We already indirectly track feeding by weighing beetles during bioassays but will also include egg counts in future observations so that we can measure changes in fecundity. We are also considering using flight mills to measure flight capability.

Acquisition of virus susceptible biotypes. One or more lab colonies of virus-susceptible biotypes are required for comparative bioassays. We originally planned to source non-CRB-G beetles during foreign exploration for OrNV isolates, but this has not happened because of COVID-19 travel restrictions. Our new plan is to import gravid females from places such as Palau, where one or more virus-susceptible biotypes are known to exist, in addition to CRB-G. Eggs from these females will be used to establish isofemale lineages and these lines will be genotyped using DNA extracted from the imported females.

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