Control of Little Fire Ant (LFA) and Coconut Rhinoceros Beetle (CRB) on Guam

May 4, 2020

Contents

1 Combined Budget

Item	Cost(UOG)	Cost(GDOA)	Total
Personnel	$66,200	$72,987	$139,187
Benefits	$19,200	$0	$19,200
Travel	$4,000	$0	$4,000
Supplies	$0	$2,545	$2,545
SUBTOTAL	**$89,400**	**$75,532**	**$164,932**
Administrative fee	$15,776	$13,329	$29,106
TOTAL	**$105,176**	**$88,861**	**$194,038**

2 Little Fire Ant Management

Please see next page.

GRANT NARRATIVE FORMAT

1. S&PF PROGRAM – Forest Health Protection

2. STATE AGENCY NAME – University of Guam, College of Applied and Natural Science

3. PROJECT COORDINATOR(S) –

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4. STATEMENT OF NEED/PURPOSE –

Little fire ant (LFA), *Wasmannia auropunctata*, was detected in November 2011 in Yigo, a northern village of Guam, at the side of a green waste repository. Subsequent LFA surveys throughout Guam found it to be established at over 35 widely dispersed sites. Previous LFA infestations in the Pacific Basin include those of the five major islands of Hawaii, New Caledonia, French Polynesia and Northern Queensland, Australia. The devastating effects of LFA on agriculture and forest ecosystems observed in these other Pacific jurisdictions are being repeated on Guam and may potentially occur on other Micronesian islands that are LFA-free. LFA's spread into and throughout Guam is due to human transport of plant related material.

The Guam Invasive Species Management Plan identifies LFA as a Priority Invasive Species (p.15) and recommendations "to seek grants and other mechanisms to provide the LFA working group with the needed tools and resources to continue control efforts and subsequently the eventual eradication of the LFA from Guam" (p.35). Additionally, the Regional Biosecurity Plan for Micronesia and Hawaii (Attachment L, Guam, p.L-12) recommends to "Improve Post Border Biosecurity" through the high priority action item to "Determine the extent of Little Fire Ant infestation and then to manage and/or eradicate this species".

The Forestry Division of Guam Department of Agriculture (GDOAG) maintains a 70-acre conservation acacia forest in Santa Rita, a southern village of Guam. The Cotal Conservation

Forest is a long-term reforestation project to return nutrients to the soil, replace acacia trees with native flora such as the endangered *Seriianthese nelsonii* and ultimately reintroduce native fauna such as the endangered Guam Rail. The forest is directly bordered on the west by residential homes. Further southwest is Department of Defense (DoD) land on which the US Naval Base Guam Munitions site is situated. LFA was identified in the residential neighborhood in 2015 and subsequent surveys by the University of Guam (UOG) showed rampant spread throughout the area. The Center for Environmental Management of Military Lands also conducted surveys in 2017 to delineate LFA infestation at the Naval Base Guam Munitions site. LFA were found at 22 sites along the northeast border of the munitions site. A preliminary survey by the DOAG-Biosecurity division in 2017 identified LFA at 33 points along walking trails throughout the forest and along the residential border. Although unconfirmed, LFA infestation is believed to have originated in the residential area from contaminated backfill soil. UOG personnel, lead Dr. Ross Miller, began survey and treatment in 6/2018 and ended in 2/2019 after depletion of funds, Figure 1. DOAG resumed treatment of ~25-acre infestation in 6/2019 as funds and personnel were made available. Survey results from 2/2020 (Figure 2) show significant progress towards eradication, yet a substantial amount of area still infested despite approximately two years of treatment.

This project proposes to continue LFA eradication efforts in the conservation forest and establish and maintain a perimeter/buffer zone to prevent further infestation from surrounding areas. Currently, an ongoing joint effort by DOAG (funded by Department of the Interior) and UOG (funded by DoD) is working to eradicate LFA in the forest. Techniques developed by the University of Hawaii-Hilo Ant Lab, that have proven effective on Hawaii, are transferrable to Guam. We utilize these methods and adapted them for aerial drone use. Application drones allow for faster and safer treatment of technically challenging areas such the forest canopy or dense impassable jungle. Significant resources have been invested in reclamation of this forest and LFA infestation compromises future restoration work. If successful, the eradication will also reduce the threat of DoD land infestation posed by LFA in the neighboring forest.

This project addresses Guam State-Wide Assessment and Resource Strategy (SWARS), Strategy 2: Protect, Conserve and Restore Forests On State, Private, And Other Nonmilitary Lands (p.103). The eradication of LFA will increase forest resiliency and conservation can be achieved by “reduce(ing) stressors to existing forest through enhancement of current stands” (p.108)

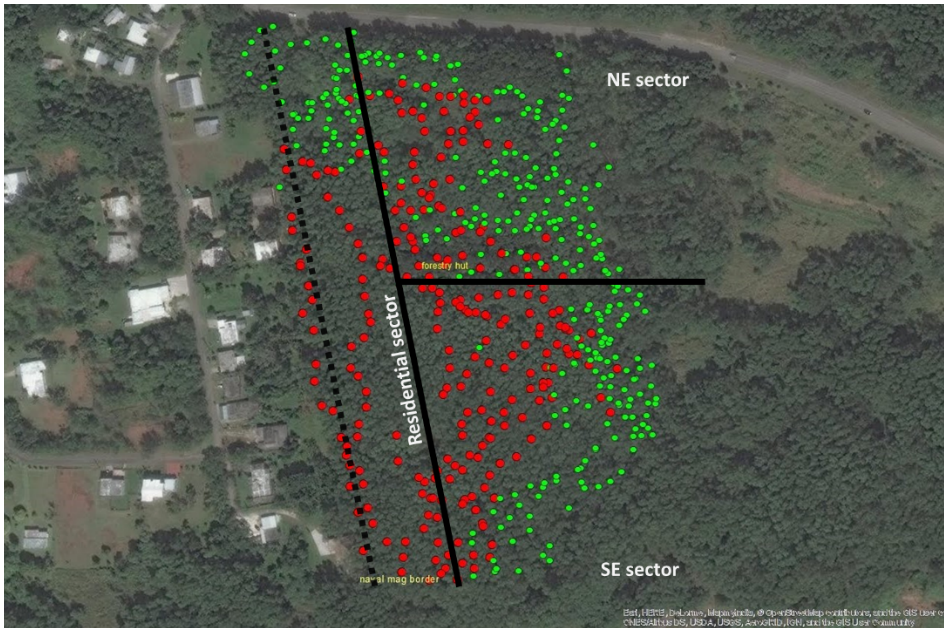

Figure 1. Initial delineation of LFA in the Cotal Conservation Forest section south of Cross Island Rd. on 6/2018. Red dots denote presence of LFA and green dots denote no LFA. Broken line represents buffer area proposed in objective 2. Data collected by UOG, edited by G.Dulla.

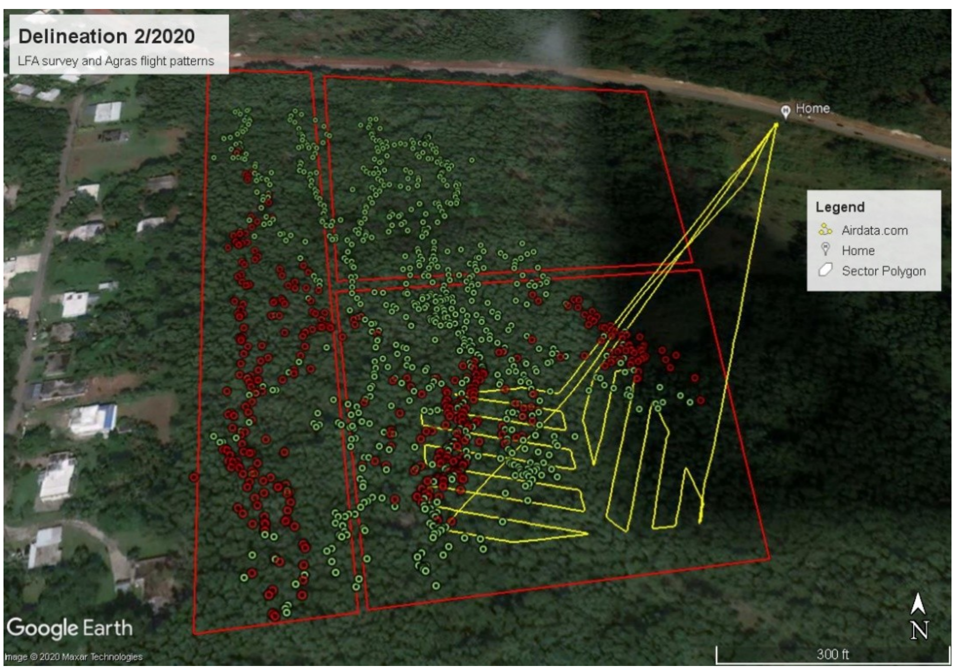

Figure 1. Delineation of LFA in the Cotal Conservation Forest section south of Cross Island Rd. on 2/2020. Red dots denote presence of LFA and green dots denote no LFA. Yellow lines are examples of aerial treatment patterns utilizing drone dispersal. Data collected by DOAG.

5. GOALS –

The project's overall goal is to eradicate LFA from the 70-acre conservation forest in Santa Rita, Guam and develop the capacity within DOAG to treat LFA on a large scale with limited personnel. This is an ongoing project to eradicate LFA from the Cotal conservation forest with working objectives to clear all LFA in the forest and prevent re-infestation through a monitored buffer and/or eradicate LFA from the adjoining residences. It is anticipated that the Biosecurity and Forestry Divisions will maintain these after the end of the funding period.

6. OBJECTIVES –

The following objectives address Guam SWARS, Strategy 2: Protect, Conserve and Restore Forests On State, Private, And Other Nonmilitary Lands (p.103). The eradication of LFA will increase forest resiliency (p.108) and conservation can be achieved by "reduce(ing) stressors to existing forest through enhancement of current stands."

1. Eradication of LFA from forest

2. Establish buffer zone around forest to prevent re-infestation

3. Provide training workshops and direct assistance for neighboring residents on LFA recognition, prevention of infestations, and treatment of LFA.

-This objective addresses the "Next Step and action: Identify a short list of likely landowners that would be willing to participate in a forest protection program". (Guam SWARS, p.111)

7. SPECIFIC ACTIVITIES –

1. Eradication of LFA from forest

Eradication of LFA from the forest requires a combination of surveying and pesticide treatment. Current routines will be maintained for the extension of this management project which includes monthly surveillance of targeted infested areas and quarterly surveillance of the ~25-acre portion of forest initially delineated and infested with LFA. Manual treatment with Tango (liquid pesticide-bait mixture@ up to 4-gallons/acre) and Siesta/Probait (granular pesticide@ 1.5lb/acre) is focused on the 6.5-acre section bordering the residential homes. Drone pesticide dispersal (granules @1.5lbs/acre: Amdro, MaxForce, Advion, Extinguish/ liquid: Tango) is utilized on the southeastern 7.2-acre sector where terrain make treatment difficult and dangerous. Treatment of the 7.9-acre northeast sector will cease on 7/2020 but continued surveying will occur quarterly.

- Progress will be measured in the reduction of LFA infested survey points or area throughout the project period.

2. Establish buffer zone around forest to prevent re-infestation

The border between the forest and the residential neighborhood is currently heavily infested with LFA. Treatment is ongoing with slow but significant progress. Once this 6.5-acre area is cleared of LFA, regular monthly surveying will continue along a 380m long by 5m wide path along the border. Treatment will be done as needed.

- Progress will be measured in the reduction of LFA infested survey points or area throughout the project period and regularity of monitoring along buffer.

3. Provide training workshops and direct assistance for neighboring residents on LFA recognition, prevention of infestations, and treatment of LFA.

To treat the root cause of the LFA infestation and potentially eliminate the perpetual need to survey and treat the buffer zone, this project proposes the teach the residents how to treat LFA on their property. Previous work has developed a residential workshop for LFA detection and treatment. There are 16 homes along the forest border and other willing residents will be offered the workshop. DOAG will provide treatment supplies, manage treatment schedules and regularly follow-up with resident households to ensure completion. Surveys will be completed by DOAG personnel.

- Success will be measured in the reduction of positive LFA survey points or area throughout the project period and regularity of resident treatment.

All activities above will be led by Ashley Toves, the current Research Associate I/field supervisor. Treatment, surveys and outreach are performed by the project coordinator, field supervisor and one or two field technicians. Grant administration and funds management will be managed by the Research Corporation of the University of Guam (RCUOG). RCUOG policies align with CRF 200 procurement services regarding micro purchase, small purchase, and competitive bidding thresholds.

8. KEY PERSONNEL –

Ashley Toves, Research Associate I, hired 6/2019.
- B.S. in Biology, Guam EPA Core pesticide certification and FAA small UAS Remote Pilot Certificate
- Current duties include fieldwork (treatment and surveys) supervision, data management, supplies procurement, human resources, website editing and drone operation.

Trevor Boykin, Research Assistant III, hired 11/2019.
- College coursework in Life Sciences, Guam EPA Core pesticide certification
- Current duties include fieldwork (treatment and surveys), outreach and education, and drone operation under supervision of licensed pilots.

9. RESPONSIBILITIES – No special responsibilities are required of the Forest Service or others.

10. MONITORING & EVALUATION –

The project coordinator is responsible for monitoring and reporting project activity. Daily activity progress will be tracked in weekly activity reports by paid employees and reviewed by project coordinator. Quarterly survey results will be mapped and the reduction of LFA positive points and infested area will be quantified. Bi-annual reports, including objective progress narratives and standard federal financial forms, will be provided to USDA-FS 30 days after reporting period.

Consultation and project review by DOAG-Forestry and USDA-FS will determine if goals and Guam SWARS #2 is adequately addressed.

11. BUDGET –

PROGRAM:

Budget Items by SF 424A Object Class Categories	Federal $	State Match $	Other Match $	Source of “Other Match”
a. Personnel				
Research Associate I, fulltime @$20.34/hour, 2080hrs	*$42,307*	*$0*		
Research Assistant III, fulltime or 2x halftime @ $14.75/hour, 2080hrs	*$30,680*	*$0*		
b. Fringe Benefits				
none	*$0*	*$0*		
c. Travel				
none	*$0*	*$0*		
d. Equipment				
none	*$0*	*$0*		
e. Supplies				
Fuel, ~7,488miles, work truck 20MPG, $4.50/gallon	*$1,685*	*$0*		
Survey Supplies; peanut butter, chopsticks, fluorescent tape/flags, ziplock bags	*$500*	*$0*		
Wrist Garmin GPS units (2x $180)	*$360*	*$0*		
f. Contractual				
none	*$0*	*$0*		
g. Construction				
none	*$0*	*$0*		
h. Other				
none	*$0*	*$0*		
i. Total Direct Charges (sum of a-h)	$75,622	*$0*		

Budget Items by SF 424A Object Class Categories	Federal $	State Match $	Other Match $	Source of “Other Match”
j. Indirect Charges (15% of total)	$11,343	*$0*		
k. Totals (i + j)	$86,965	*$0*		
l. Program Income	*$0*	*$0*		

3 Coconut Rhinoceros Beetle Biological Control

Please see next page.

Grant Proposal: USDA Forest Service FY2020

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

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University of Guam College of Natural and Applied Sciences

May 4, 2020

Contents

1. Project Description

This grant proposal is a request for funds to extend employment of an insect pathologist, Dr. James Grasela, for one year to work on the Guam CRB Biological Control Project, a multiyear project partially funded by USDA-APHIS and Department of the Interior Office of Insular Affairs.

The specialized knowledge and skills of an insect pathologist are essential to success of this project. Dr. Grasela has identified two biological control agent candidates for CRB-G (both are isolated of *Oryctes* nudivirus (OrNV)). If funded, the proposed grant will support the insect pathologist's participation in the following activities.

Objective 1: Survey to Determine Background OrNV Incidence A pre-release survey will be performed to measure the incidence of OrNV in the Guam CRB-G population using PCR.

Objective 2: Establish Sustainable CRB-G Biocontrol by Autodissemination of OrNV OrNV isolates which have been identified as biological control agents will be propagated *in vivo* and introduced into the Guam CRB-G population via auto-dissemination under conditions specified by an existing USDA-APHIS permit.

Objective 3: Establish Island-wide Monitoring Systems for CRB and Coconut Palm Health Incidence of OrNV infection in beetles caught by pheromone traps will be monitored using PCR.

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 replanting of oil palm plantations. If a CRB population outbreak is not suppressed, it is possible for initiation of a positive feed-back cycle where 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, Rota, 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; Secretariat of the Pacific Community 2017).

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 B: *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

This proposal is, in part, a response to an urgent need expressed by Micronesian Island leaders in the 22nd Micronesian Islands Forum Communique (Appendix C):

> 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.

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).

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**.

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. Specialized skills of an insect pathologist are essential to implementing successful biological control of CRB-G on Guam and elsewhere.

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

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.

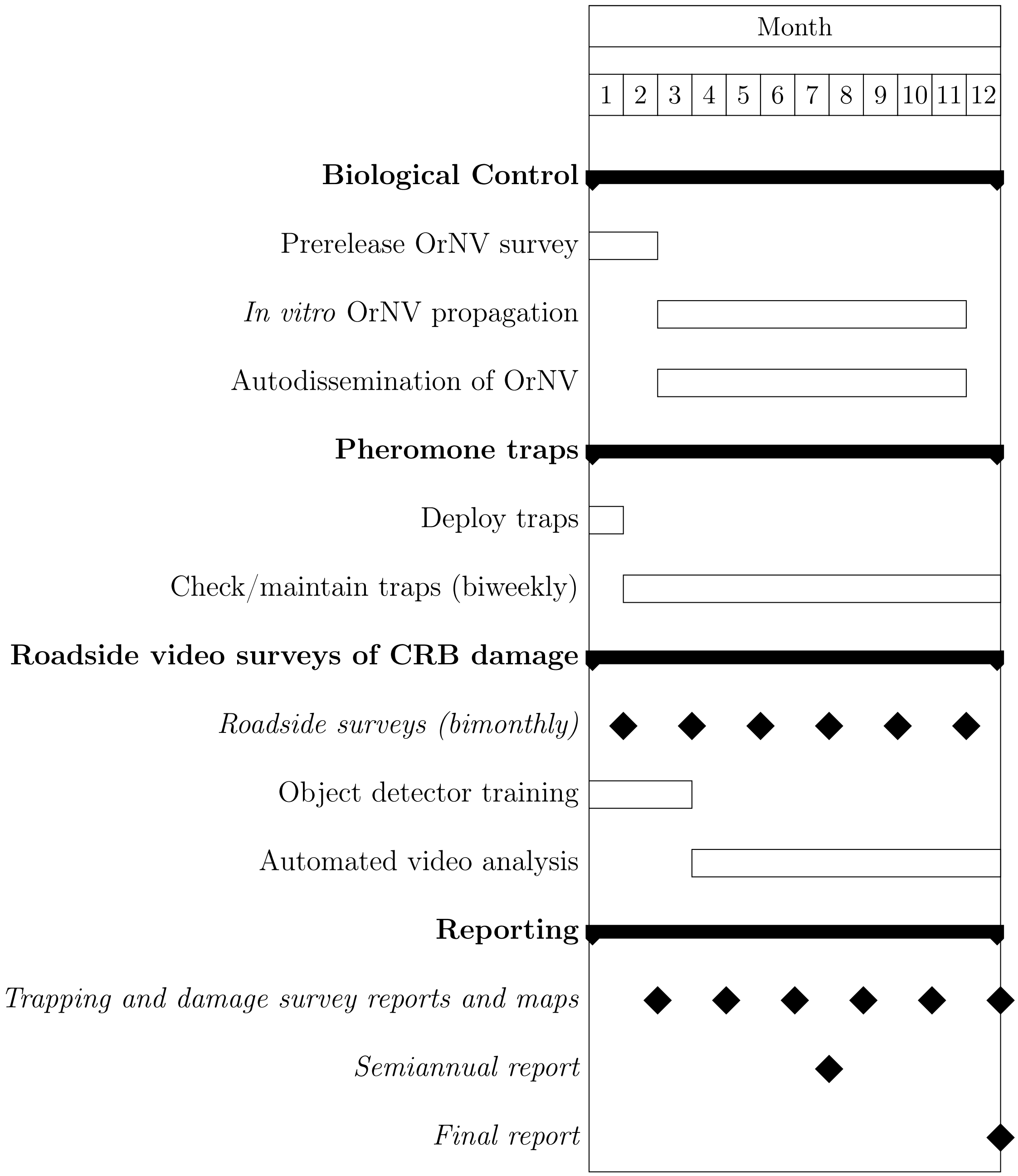
Pheromone Traps We plan to installed 150 CRB pheromone monitoring traps. These will be baited with oryctalure and serviced semimonthly. These traps catch approximately equal numbers of males and females which remain alive in the traps for several weeks. Collected beetles will be used for autodissemination 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 (See Supporting Technical Data 2). 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


Month
1
2
3
4
5
6
7
8
9
10
11
12
Biological Control
Prerelease OrNV survey
In vitro OrNV propagation
Autodissemination of OrNV
Pheromone traps
Deploy traps
Check/maintain traps (biweekly)
Roadside video surveys of CRB damage
Roadside surveys (bimonthly)
Object detector training
Automated video analysis
Reporting
Trapping and damage survey reports and maps
Semiannual report
Final report

1.5. Potential Benefits

- This project will directly benefit Guam. Without implementation of effective biological control, it is likely that 50% or more coconut palms will be killed by CRB-G. 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 with 39% the trees in Guam's forests are at risk of attack by CRB-G. If the 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).

- This project will indirectly benefit all other islands in Micronesia and other areas of the Pacific. With very high populations of CRB-G in Gaum risk of accidental introduction to other islands is extremely high. CRB-G has already been intercepted twice on Saipan, it is established on Rota and it has been detected on Aguiguan. If CRB-G infests smaller islands and atolls in the FSM and RMI where the coconut palm as the *tree of life*, islanders may have to migrate to larger population centers. If funded, the proposed project will be run under the *Open Science* concept. All data and analyses will be publicly shared on the internet in near-real time and samples of biocontrol candidates will be also be shared other Pacific islands battling CRB-G.

- It is expected that establishment of OrNV as an effective biological control agent will provide permanent self-sustaining population suppression of CRB-G. This is in contrast to temporary results from invasive species control programs which rely on population suppression using pesticide application, trapping or physical removal.

- Technology developed for automated roadside video surveys may be used for early detection of CRB on islands on which this pest has not yet been detected. Roadside videos may be of use for monitoring spread of invasive weeds such as Mexican creeper, *Antigonon leptopus*.

2. Budget

Item	Cost
Personnel	$66,200
Benefits	$19,200
Travel	$4,000
SUBTOTAL	**$89,400**
Administrative fee	$13,410
TOTAL	**$102,810**

Personnel includes salary for an insect pathologist (Dr. James Grasela, 1 FTE, $64,000) and benefits calculated at 30% * salary. Also includes PI's salary compensation calculated at 0.02 FTE * $110,000.

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

Administrative fee equal to 15% 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

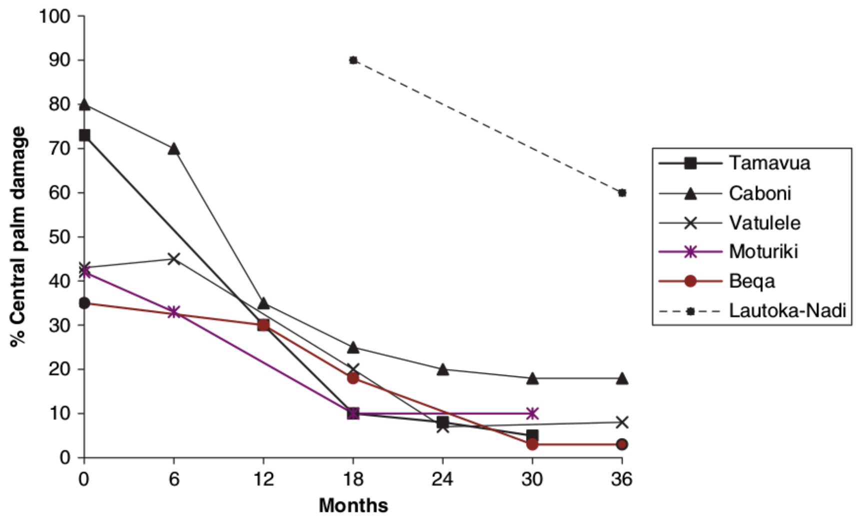

Figure 1: Reduction in coconut palm damage following release of *Oryctes rhinoceros* nudivirus in Fuji. (Jackson 2009)

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

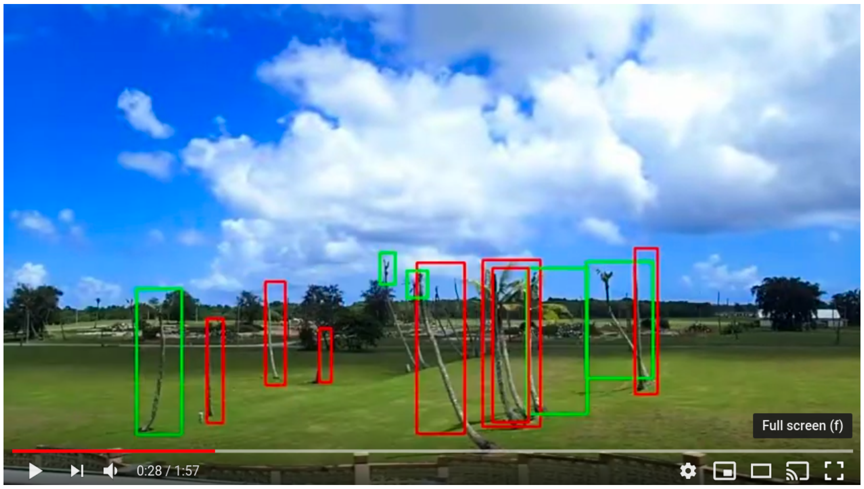

Figure 2: Training an Object Detector to Locate Coconut Palms Damaged or Killed by Coconut Rhinoceros Beetle. https://youtu.be/zzSorqcmt9U.

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. 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.

Contents

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

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

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.

- **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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