Biological Control of Coconut Rhinoceros Beetle Biotype G in Micronesia

Aubrey Moore, University of Guam

February 21, 2018

Contents

1 Project Initiation

- This project was initiated by an award letter transmitted from Assistant Secretary Nikolao Pula to Governor Eddie Baza Calvo dated July 21, 2017 (Appendix A: Award Letter). The letter awards $176,516 to the University of Guam "for the College of Agriculture and Life Sciences[1] to hire a post-doctoral entomologist for 2 years to work on an existing , funded project to implement effective biological control for the coconut rhinoceros beetle (CRB) which is rapidly killing palm trees on Guam and Palau as detailed in the proposal dated July 5, 2017 (Appendix B: Proposal). The funding period for this project is September 1, 2017 through October 31, 2019.

- Project and finances were established under the Research Corporation of the University of Guam.

2 Recruitment

- A job description and position announcement for a post-doctoral insect pathologist was written (Appendix C: Post-doctoral Entomologist Position Announcement) and posted on the University of Guam web site and on the Entomological Society of America web site.

3 Research Progress

- Permit applications to allow importation of *Oryctes rhinoceros* nudivirus (OrNV) isolates were submitted to USDA-APHIS and the Guam Department of Agriculture. These were approved. The first isolate to be imported under these permits was collected from CRB Guam biotype adult beetle caught in the Dumaguete area of Negros Island, Philippines during field work conducted there by Dr. Aubrey Moore (UOG), Ian Iriarte (UOG), and Dr. Sean Marshall (AgResearch, New Zealand). The isloate was cleaned up and propagated at AgResearch New Zealand prior to being sent to Guam for pathogenicity testing.

- Field collected CRB-G adults which were challenged with a high dose of OrNV showed no significant response (Technical Report: Bioassay of OrNV Isolated from CRB-G, Negros Island, Philippines).

- An environmental cabinet which will allow control of temperature, relative humidity, and lighting during bioassays was procured and installed.

[1] The correct name is "College of Agriculture and Natural Sciences".

4 Appendix A: Award Letter

See following page.

United States Department of the Interior

OFFICE OF INSULAR AFFAIRS
1849 C Street, NW
Washington, DC 20240

Assistant Secretary

JUL 21 2017

The Honorable Eddie Baza Calvo
Governor of Guam
Ricardo J. Bordallo Governor's Complex
Adelup, Guam 96910

Dear Governor Calvo:

I am pleased to award funding to the Government of Guam through the Office of Insular Affairs' Coral Reef/Natural Resources Program. Funds will be used for the following invasive species control and eradication efforts for the Coconut Rhinoceros Beetle and Little Fire Ant:

- $74,516 for the Department of Agriculture for an outreach program for prevention, control and management of the Little Fire Ant infestation on Guam as detailed in the proposal dated July 3, 2017;

OIA is also awarding funding to the University of Guam for the following projects:

- $176,553 for the College of Agriculture and Life Science to hire a post-doctoral entomologist for 2 years to work on an existing, funded project to implement effective biological control for the coconut rhinoceros beetle (CRB) which is rapidly killing palm trees in Guam and Palau as detailed in the proposal dated July 5, 2017; and

- $63,816 for the College of Natural & Applied Sciences to hire a field technician and purchase supplies and equipment to eradicate the little fire ant infestation throughout the island. This project will also enhance ongoing cooperation between the University of Guam, the National Park Service, the Department of Defense and the Government of Guam as detailed in the proposal dated July 3, 2017.

The grant award documents will be sent to your staff by our grant manager. Please contact me or have your staff contact Tanya Joshua at (202) 208-6008 or tanya_joshua@ios.doi.gov, point of contact for the Coral Reef/Natural Resources Program, should you have any questions on concerns regarding these grants.

We look forward to working with you and your staff to implement these grants.

Sincerely,

Nikolao Pula
Acting Assistant Secretary
for Insular Areas

cc: President Tommy Remengesau Jr
President Peter Christian
President Hilda Heine
Governor Johnson Elimo
Governor Ralph DLG. Torres
Governor Marcelo Peterson
Governor Lyndon Jackson
Lt. Governor James Yangetmai
Congresswomen Madeleine Bordallo
Congressman Gregorio Kilili Camacho Sablan
Dr. Robert Underwood
Jaimie Reaser, PhD, Executive Director NISC

5 Appendix B: Proposal

See following page.

Biological Control of Coconut Rhinoceros Beetle Biotype G in Micronesia

Aubrey Moore, University of Guam

July 7, 2017

Contents

1. Project Description

This grant proposal is a request for funds to hire a post doctoral entomologist for 2 years to work on an existing project to implement effective biological control for the coconut rhinoceros beetle (CRB) which is rapidly killing palm trees in Guam and Palau. Without significant population suppression of CRB in Guam and Palau, it is likely that Guam and Palau will lose most of their palms and it is just a matter of time before other Micronesian Islands are invaded by CRB.

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 is not suppressed, it is possible for a positive feed-back cycle to initiate whereby 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 [1]. A similar outbreak 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), and Honiara, Solomon Islands (2015). 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)[2]. 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 [3] . OrNV attacks only CRB, typically reducing damage by up to 90% with population suppression lasting indefinitely [4]. 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 suppresses CRB populations to levels that result in only minor damage.

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 [5]. Augmentative release of OrNV continues to be an important mechanism for CRB

management in both coconut and oil palm growing regions. For ~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) [6, 7].

Recent analysis of DNA from an ongoing survey has detected CRB-Guam in Guam, 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 to all available isolates of OrNV.

Uncontrolled infestations of CRB-G may kill most palms within a few years and risk of accidental spread to other islands is high. A worse case scenario 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 (such as Typhoon Dolphin which visited Guam in May, 2015). Very high feeding activity will kill mature coconut palms, leaving standing dead coconut trunks that are ideal breeding sites for subsequent generations of beetles.

During a 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 the coconut palm is referred to as *the tree of life*. Here, 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 the spread of CRB-Guam cannot be controlled. If CRB-G infests islands and atolls where the coconut palm is the *tree of life*, islanders may have to migrate to larger population centers.

Recommended response to CRB-G outbreaks. 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 [8, 9, 10]. In a special meeting on CRB-G at the XXVth International Congress of Entomology [11], 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 center of origin.

2. Survey CRB-G populations from the cent re 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.

Once an outbreak has been suppressed by OrNV biocontrol, it is essential to preform sanitation (destruction of active or potential breeding sites) to prevent local resurgence of rhino beetle populations [12].

Recent progress. A regional CRB-G biocontrol project has not yet been established because funding sources have not been identified. However, a USDA-APHIS funded collaboration between the University of Guam and AgResearch New Zealand has made progress on all 3 objectives:

1. DNA samples from CRB populations collected in Asia indicate the presence of the CRB-G biotype in the Philippines and Indonesia.

2. During a UOG/AgResearch expedition to Negros Island, Philippines in January 2017, about 100 DNA samples from a known CRB-G population were collected.

3. Lab tests in at AgResearch New Zealand indicated that all sampled beetles belonged to the CRB-G biotype and that one of these was infected with OrNV. The OrNV from this beetle was isolated and propagated insect cell culture.

We are hoping that this new OrNV isolate will kill CRB-G adults, to be determined in bioassays to be performed on Guam. We are currently waiting for APHIS to issue an import permit to allow shipment of a sample of the new isolate from New Zealand to Guam. If the isolate proves pathogenic for CRB-G, we will immediately start *in vivo* propagation and auto-dissemination.

If OrNV biocontrol is successful, palm mortality will stop, the virus will sustainably suppress CRB damage to tolerable levels, and risk of accidental spread to other islands will be significantly reduced. This scenario is based on reports of successful implementation of CRB biocontrol using OrNV on all Pacific Islands on which the virus was released prior to discovery of the OrNV resistant biotype, CRB-G, on Guam [13].

1.2. Statement of Need

This proposal requests funds to hire a post doctoral entomologist for 2 years to implement effective biocontrol for CRB. Technical/scientific assistance in the form of a post doc is necessary because professional capacity to handle major entomological problems in Micronesia is inadequate. During the past 2 decades the number of PhD level entomologists practicing in Micronesia decreased from 9 (5 in Guam, 3 in CNMI, 1 in Palau) to 3 (all in Guam). During this same period the workload increased, mainly because

the detection rate for invasive species went up by almost an order of magnitude. The three remaining PhD level entomologists in Micronesia do not have time and resources to adequately respond to concurrent invasions of coconut rhinoceros beetle, little fire ant, Asian cycad scale, and several major agricultural pest insects. Teaching and administrative responsibilities do not allow enough time to be dedicated to finding solutions to the major entomological problems currently impacting Micronesia. The post doctoral entomologist will work under supervision of the PI within an existing CRB biocontrol program funded by USDA-APHIS.

Funding of the current proposal will enable immediate extension of benefits from the Guam biocontrol project to Palau. Island leaders are worried about the high risk of CRB-G spreading within Micronesia. A regional project with the objective of developing effect biological control for CRB was identified as an urgent need by Micronesian Governors and Presidents at the 22nd Micronesian Islands Forum which took place on Guam during May, 2017 (Appendix B). Hiring a post doc entomologist will partially address this urgent need.

1.3. Goals and Objectives

The post doctoral entomologist will work on an existing project to implement effective biological control for the coconut rhinoceros beetle, Guam biotype (CRB-G) which is rapidly killing palm trees in Guam and Palau. The overall objective of this project is establish a self-sustaining biological control which will stop palm tree mortality and eventually suppress the CRB to minimize damage to tolerable levels.

Details for the following goals can be found in the approved APHIS project work plan attached as Appendix A.

1.3.1. Perform lab bioassay to determine pathogenicity of the new OrNV isolate from Negros Island, Philippines

If the new OrNV isolate is not pathogenic for CRB-G, further foreign exploration will be necessary.

1.3.2. Establish an island-wide coconut palm health survey

A semiannual health survey will be established to quantify CRB damage to coconut palms on an island-wide basis. This survey needs to be started prior to auto-dissemination of OrNV or other control tactic is implemented so that results, in terms of reduction in CRB palm damage, can be quantified.

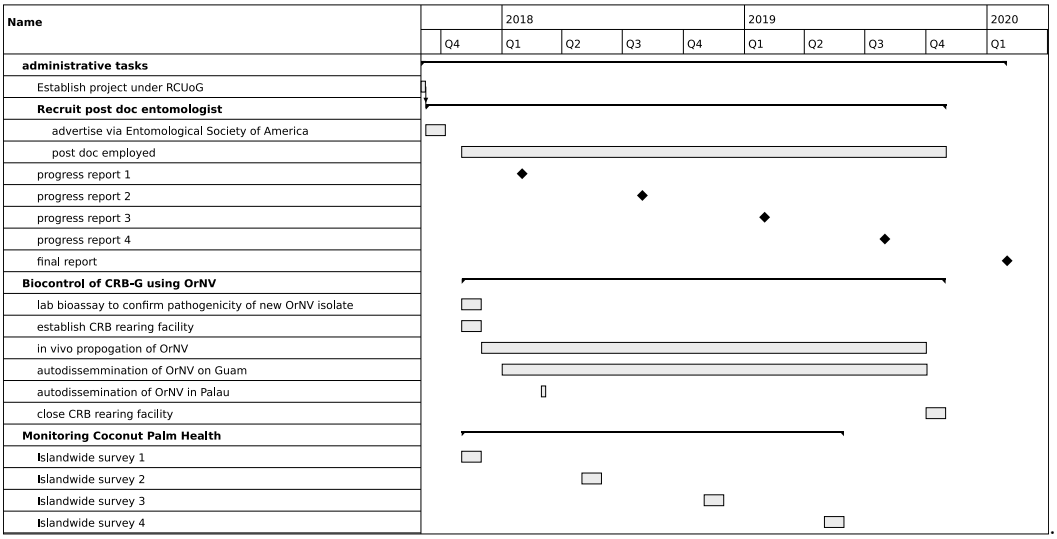
1.3.3. Establish a CRB rearing facility to provide adults

Field collected CRB will be reared in environmental rearing cabinets for use in bioassays, *in vivo* propagation of OrNV and auto-dissemination. One rearing cabinet has been ordered. Two more will be ordered with FY17 Farm Bill funds.

1.3.4. Establish biological control of CRB-G by auto-dissemination of OrNV in Guam and Palau

CRB-G adults will be used to autodisemminate OrNV in Guam and Palau.

1.4. Timeline



1.5. Potential Benefits

- This project will directly benefit Guam and the Republic of Palau. Both of these jurisdictions are infested with CRB-G. Without implementation of effective biological control, it is likely that 50% or more coconut palms will be killed by CRB-G. If the OrNV biocontrol project is successful, palm mortality will be stopped and the virus will sustainably suppress CRB damage to tolerable levels.

- This project will indirectly benefit all other islands in Micronesia. With very high populations of CRB-G in Guam and the Republic of Palau, risk of accidental introduction to other islands is extremely high. CRB-G has already been intercepted twice on Saipan, Commonwealth of the Northern Mariana Islands. 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. If the OrNV biocontrol project is successful, the risk of accidental transport of CRB-G to other islands will be significantly reduced.

2. Budget

Item	Details	Cost
Personnel		
Salary	2 yrs * $60k per yr	$120,000
Benefits	18% * Salary	$21,600
Travel		
Relocation expenses (incoming)		$6,000
Relocation expenses (outgoing)		$5,000
Trip to Palau		$6,298
SUBTOTAL		$158,898
Administrative fee	10% of total	$17,655
TOTAL		$176,553

Salary and Benefits are for employment of a PhD.D. level entomologist to work exclusively on biological control of coconut rhinoceros beetle biotype G in Micronesia under supervision of the PI.

Relocation expenses include airfare, shipment of household items, and other associated expenses such as immigration fees. $1,000 is allotted for short-term hotel accommodation upon arrival.

Trip to Palau The purpose of this trip by the PI and postdoc is to perform the initial release an effective isolate of OrNV in Palau for biocontrol of coconut rhinoceros beetle. Airfare: Guam-Palau return; 2*$769=$1538. Per diem (US State Dept. rate): 2*7d*$340=$4760. Total trip estimate=$1538+$4760=$6,298

Administrative fee A fee equal to 10% of the total grant award is charged by the Research Corporation of the University of Guam for services provided.

3. Contact Information

3.1. Grant Recipient

Name Dr. Lee S. Yudin

Title Dean / Director, College of Natural and Applied Sciences, University of Guam

Address Agriculture and Life Sciences Bldg, 303 Campus Dr., University of Guam, Mangilao, Guam 96923

Telephone (671) 735-2000

Email lyudin@triton.uog.edu

3.2. Grant Manager

Name Dr. Aubrey Moore

Title Entomologist, College of Natural and Applied Sciences, University of Guam

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Telephone (671) 686-5664

Email aubreymoore@guam.net

3.3. Automated Standard Application for Payments (ASAP)

ASAP Location Code 14010001

ASAP ID 6637846

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4. APPENDICES

A. Work Plan for CRB-G Biocontrol Project Funded by USDA-APHIS

Farm Bill Work Plan - Fiscal Year 2017

Cooperator:	**University of Guam**			
State:	Guam			
Project:	Oryctes Nudivirus for Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle			
Project funding source:	Farm Bill Section 10007			
Project Coordinator:	Aubrey Moore			
Agreement Number	17-8515-2058-CA			
Contact Information:	**Address:**	303 University Drive, Room 105 Agriculture and Life Sciences Building College of Natural & Applied Sciences University of Guam Mangilao, Guam 96923		
	Phone:	671-686-5664	**Fax:**	na
	Email Address:	aubreymoore@guam.net		

This Work Plan reflects a cooperative relationship between the University of Guam (the Cooperator) and the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ). It outlines the mission-related goals, objectives, and anticipated accomplishments as well as the approach for conducting a project entitled **Oryctes Nudivirus for Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle** and the related roles and responsibilities of the parties [e.g., APHIS role(s) and Cooperator role(s)] as negotiated.

I) OBJECTIVES AND NEED FOR ASSISTANCE

The population of coconut rhinoceros beetles (CRB) recently established on Guam is genetically distinct from other populations of this major palm pest and it is being referred to as the CRB-G biotype. CRB-G is resistant to Oryctes nudivirus (OrNV), which is the major biocontrol agent for CRB, and it appears to have other characteristics, which make it more invasive and harder to control than other CRB biotypes. While there were no range expansions of CRB for a quarter of a century (1980 to 2005), CRB is now on the move with the 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. It is significant that all of these new invasions involve CRB-G. Thus, CRB-G is a regional problem, with Guam currently experiencing massive mortality of coconut palms as the result of a CRB population explosion triggered by abundant larval breeding sites left in the wake of a recent typhoon.

The objective of this project is to stop an uncontrolled outbreak of coconut rhinoceros beetle biotype G which is rapidly killing palms on Guam. Entomologists working on this problem agree that the most feasible solution is establishment of biological control using an isolate of OrNV which is highly pathogenic to CRB-G.

Financial assistance will facilitate:

1. continued support of an international collaborative project with the goal of discovering a strain of OrNV or other microbial biocontrol agent which is highly pathogenic for CRB-Guam
2. continued support for a graduate research assistant at the University of Guam
3. continued support for operating an insect pathology laboratory at the University of Guam to evaluate candidate biocontrol agents discovered during foreign exploration
4. support for a semiannual island-wide coconut palm health survey for Guam

Items 1, 2 and 3 are currently supported by my FY2016 Farm Bill (performance period: August 1, 2016 through July 30, 2017). Item 4, which establishes a semiannual coconut palm tree health survey, is a new activity.

This project is aligned with FB goal 6: Enhance Mitigation and Rapid Response.

II) RESULTS OR BENEFITS EXPECTED

- Foreign exploration leading to discovery of a highly pathogenic strain of OrNV or other microbial biocontrol agent for CRB-Guam could lead to implementation of self-sustaining population suppression and tolerable damage levels on Guam and other islands invaded by CRB-G.
- Loss of 50% or more of Guam's palms may be prevented if an effective biocontrol agent is found and released quickly.
- Reduction in CRB population levels on Guam will reduce the risk of accidental introduction of the highly invasive CRB-Guam biotype to other Pacific islands and elsewhere.
- Development of image analysis methods may lead to a small, inexpensive, automated CRB damage detector which could be mounted on a drone or a conventional vehicle. This device could be used for early detection or monitoring of CRB damage.

III) APPROACH

1. "Witch's Brew" Bioassays

In previous years, we tested several isolates of OrNV from AgResearch New Zealand and some from virus-infected beetles in Fiji. We did not observe significant mortality during many bioassays, leading us to the conclusion that CRB-G is resistant to OrNV. However, to confirm that we do not have OrNV pathogenic for CRB-G, we have started a series of "witch's brew" bioassays. Frozen, dead beetles from all previous bioassays were added to one liter of water and made into an aqueous slurry using a blender. Vials containing remnants of virus samples from AgResearch New Zealand were agitated in 500 ml of water, and this suspension was added to the blender. The slurry was poured into a small pail and forty beetles were made to swim in this for thirty minutes. A control group of beetles was made to swim in water for thirty minutes. Beetles were kept in a large container filled with moist, commercially blended steer manure and soil. All beetles were checked weekly. Dead beetles were recorded and frozen.
We found a significantly higher mortality in beetles which swam in the slurry as opposed to beetles which swam in water. We made a fresh "witch's brew" by blending all dead beetles from this assay, and again observed mortality significantly higher than that of the control group. After 4 cycles of this experiment, mortality in the treatment group continues to increase. We will continue these witch's brew experiments and send beetle tissue samples to AgResearch New Zealand to test for OrNV.

2. Regional Collaboration on CRB-G Management

Moore will continue to work with collaborators at AgResearch New Zealand and the Secretariat of the Pacific Community (SPC) to put together a regional collaboration with the objective of finding an effective biocontrol agent for CRB-G.

3. Foreign Exploration for an Effective Biocontrol Agent for CRB-G

During January, 2017, Moore, Iriarte and Marshall did field work on Negros Island, Philippines, were CRB-G coexists with other CRB biotypes. The major objective was to find an effective biocontrol agent for CRB-G and a secondary objective was to develop and test protocols for further foreign exploration. DNA analysis of CRB and OrNV from rhino beetle gut samples collected during the trip is being done by Dr. Sean Marshall in his lab at AgResearch New Zealand. Bioassays of any detected OrNV will be done at the University of Guam.

Further foreign exploration for an effective biocontrol agent for CRB-G is contingent on results from this first expedition. One additional trip to the Philippines by Moore, Marshall and Iriarte is planned. Moore and Iriarte will travel to Marshall's AgResearch lab in Christchurch for training in propagation of OrNV.

4. Coconut Palm Health Survey

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. We intend to establish a semiannual coconut tree health survey rather than re-establish pheromone trapping.

4.1 Survey Method

The Coconut Palm Health Survey will use the following methodology to track changes in levels of damage caused by CRB-G.

- The survey will monitor at least 1,000 palms located throughout the island. An aluminum tag with a unique identifier will be affixed to each palm on the initial visit.
- The free smart phone app, EpiCollect+ will be used to georeference each palm, record a digital image, and record damage data. (We have successfully used this free app for several localized palm health surveys.)
- The survey will be performed twice per year.
- CRB damage will be recorded in 3 boolean data fields:
    - Mortality: 1 if palm is dead; 0 otherwise
    - New damage: 1 if any of the 4 youngest fronds have V-shaped cuts; 0 otherwise
    - Old damage: 1 if any other fronds have V-shaped cuts; 0 otherwise

4.2 Digital Image Analysis

We propose to add a methods development component to the survey. CRB damage symptoms in the form of V-shaped cuts in fronds are distinctive and easy to see in digital images. Digital imagery has been used for detection and monitoring of CRB. For example, Soloman Sar in Papua New Guinea has developed a Rapid Damage Assessment System in which geotagged images of palms are rated for damage severity.

It may be possible to automate detection and monitoring of CRB damage by training a computer to detect V-shaped cuts in digital images of coconut palms. We will test this idea using human classified image libraries as training sets. If successful, we will program a Raspberry Pi 3 equipped with a camera to detect and quantify CRB damage in real time. This small, inexpensive CRB damage detector could be mounted on a drone or a conventional vehicle for automated detection and monitoring of CRB and damage caused by this pest.

A. **The Cooperator will:**

1. By function, what work is to be accomplished?

The cooperator will perform activities outlined above.

2. What is the quantitative projection of accomplishments to be achieved?

This project is based on contingencies which preclude projection of an exact timeline.

- The first goal of this project is to find an effective biological control agent for CRB-G. This will most probably be an isolate of OrNV either discovered during foreign exploration or selected for within the "Witch's Brew" bioassays.
- The PI will apply for renewal of an existing APHIS-PPQ permit to import and release OrNV so that candidate isolates can be released soon after discovery and completion of initial lab bioassays.

b. What criteria will be used to evaluate the project? What are the anticipated results and successes?

The semiannual coconut palm health survey will be initiated during the first 6 months of the project so that a reduction in tree mortality and defoliation in response to biocontrol agent releases can be measured.

Success of the project will be evaluated by large reductions in tree mortality and defoliation by CRB-G.

3. What numbers and types of personnel will be needed and what will they be doing?

The PI and his grad student will perform biocontrol work and damage surveys on Guam. Dr. Sean Marshall, under a contract between UOG and Ag Research New Zealand, will perform DNA analysis of CRB and OrNV. He will also propagate candidate OrNV isolates in insect cell culture.

4. What equipment will be needed to perform the work? Include major items of equipment with a value of $5,000 or more.

The project will require a service vehicle for the island-wide coconut palm health survey. This vehicle will be provided by the University of Guam.

Two incubators with temperature and humidity control will be purchased for rearing coconut rhinoceros beetles for bioassays and auto-dissemination. We will purchase the same units that are being used for CRB rearing at the

University of Hawaii so that we can share methodology. These Percival Scientific Biological Incubators Model I-41LLVL. These units are quoted at $17,500 each, including shipping to Guam.

Identify information technology equipment, e.g., computers, and their ancillary components. All information technology supplies (e.g., small items of equipment, connectivity through air cards or high speed internet access, GPS units, radios for emergency operations etc.) should be specifically identified.

- Android smart phones will be used for the island-wide coconut palm health survey.
- Raspberry PI 3 computers equipped with HD cameras will be used for digital image analysis. Five units will be required for the project.
  - Computers: Raspberry atteryPi 3 (RPi3) Model B Quad-Core 1.2 GHz 1 GB RAM, On-board WiFi and Bluetooth Connectivity
  - Cameras: 5 megapixel native resolution sensor-capable of 2592 x 1944 pixel static images
  - Accessories: battery pack, realtime clock, case, microSD cards

5. **What supplies will be needed to perform the work?**

   Supplies will include containers and rearing media for CRB to be used in laboratory bioassays.

6. **What procurements will be made in support of the funded project and what is the method of procurement (e.g., lease, purchase)?** (Cooperator procurements shall be in accordance with OMB Circulars A-102 or A110, as applicable.)

   Two Android smart phones will be used for the island-wide coconut palm health survey. These will be leased under a service plan from a local telecommunications provider.

   Two incubators with temperature and humidity control will be purchased for rearing coconut rhinoceros beetles for bioassays and auto-dissemination. We will purchase the same units that are being used for CRB rearing at the University of Hawaii so that we can share methodology. These Percival Scientific Biological Incubators Model I-41LLVL. These units are quoted at $17,500 each, including shipping to Guam.

7. **What are the travel needs for the project?**

   - Local travel for coconut palm health surveys

- Foreign exploration for an effective biocontrol agent for CRB-G will be done in the Philippines where a population of the CRB-G biotype has been located.
- Progress on this project will require the PI and his grad student to visit Dr. Sean Marshall's lab in Christchurch, New Zealand to learn OrNV propagation technique.

8. Reports:
Submit all reports to the APHIS Authorized Department Officer's Designated Representative (ADODR). Reports include:

a. Narrative accomplishment reports in the frequency and time frame specified in the Notice of Award, Article 4.

b. Federal Financial Reports, SF-425 in the frequency and time frame specified in the Notice of Award, Article 4.

Are there any <u>other</u> contributing parties who will be working on the project?

- The Guam Department of Agriculture will be recruited to assist with the island-wide coconut palm damage survey.
- Dr. Sean Marshall, AgResearch New Zealand will perform DNA analysis of CRB and OrNV and he will characterize and propagate isoltes which are candidates for CRB-G biocontrol.

B. APHIS Will:

1. Outline the Agency's (USDA APHIS PPQ) substantial involvement.

a. Include any significant Agency collaboration and participation

- Provide input and oversight in the development and execution of the survey to ensure it meets national program goals and APHIS mission needs.
- Provide funds to the cooperator to cover costs as outlined in the financial plan.
- Provide additional guidance and/or technical assistance to the project coordinator, as requested.
- Assist in clarifying survey methods and detection, as well as, identification resources, as needed.
- Support the work and financial plan development by the cooperator.

b. Project oversight and performance management

- Notify the project coordinator of reporting deadlines.
- Provide guidance in the compilation and submitting of reports and other administrative matters.
- Maintain data spreadsheets showing due dates for reports, requests for allocation, forms submitted, tracked by the survey specialist.
- Provide general oversight and quality assurance of the program.

c. Provide the equipment requested by the cooperator in 4.b. & c.

d. Provide the supplies requested by the cooperator in 6.b. & c.

IV) GEOGRAPHIC LOCATION OF PROJECT

Laboratory work will be done at the University of Guam, Mangilao, Guam and at AgResearch, Christchurch, New Zealand. Foreign exploration for an effective biological control agent for CRB-G will be done in the Philippines and possibly other locations. Coconut palm health surveys will be performed on Guam.

V) DATA COLLECTION AND MAINTENANCE

All data and technical reports generated by this project will be immediately available on-line in an Open Science Framework project entitled "CRB-G Management" at https://osf.io/5js9z/.

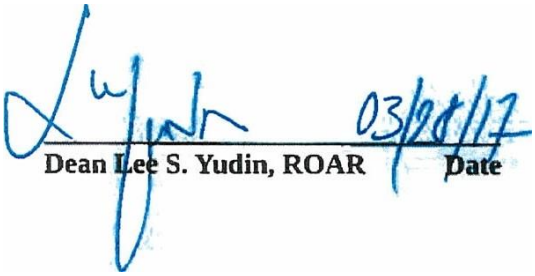
VI) TAXONOMIC SUPPORT

Taxonomic support is not required for this project.

VII) SURVEY SUMMARY FORM

Not applicable because this project does not include a pest survey.

VIII) SIGNATURES



B. 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

EDDIE BAZA CALVO
GOVERNOR OF GUAM
Chairman/Host

PETER CHRISTIAN
PRESIDENT OF THE FEDERATED STATES OF MICRONESIA
Member

TOMMY REMENGESAU, JR.
PRESIDENT OF THE REPUBLIC OF PALAU
Member

HILDA HEINE
PRESIDENT OF THE REPUBLIC OF THE MARSHALL ISLANDS
Member

RALPH D.L.G. TORRES
GOVERNOR OF THE COMMONWEALTH OF THE NORTHERN MARIANA ISLANDS
Member

JOHNSON ELIMO
GOVERNOR OF CHUUK
Member

LYNDON H. JACKSON
GOVERNOR OF KOSRAE
Member

MARCELO PETERSON
GOVERNOR OF POHNPEI
Member

JAMES YANGETMAI
LT. GOVERNOR OF YAP
Member

22nd Micronesian Islands Forum

MAY 01, 2017 ~ MAY 03, 2017 | GUAM

MAY 0 2 2017

EDDIE BAZA CALVO
Governor
U. S. Territory of Guam

TOMMY E. REMENGESAU, JR.
President
Republic of Palau

PETER M. CHRISTIAN
President
Federated States of Micronesia

HILDA C. HEINE, Ed. D
President
By Minister John M. Silk
Republic of the Marshall Islands

JOHNSON ELIMO
Governor
Chuuk State, FSM

RALPH D.L.G. TORRES
Governor
Commonwealth of the
Northern Mariana Islands

MARCELO K. PETERSON
Governor
Pohnpei State, FSM

for
LYNDON H. JACKSON
Governor
Kosrae State, FSM

JAMES YANGETMAI
Lt. Governor
Yap State, FSM

C. Cover Letter

UNIVERSITY OF GUAM
UNIBETSEDÅT GUAHAN

Aubrey Moore, PhD
College of Agriculture and Life Sciences
University of Guam
Magilao, Guam 96923

July 5, 2017

Nikolao Pula, Director
Office of Insular Affairs
U.S. Department of the Interior
1849 C Street, N.W. Mail-Stop 2429
Washington, D.C. 20240

Dear Director Pula,

I am pleased to submit a grant proposal for consideration under the little fire ant/coconut rhinoceros beetle funding opportunity you announced to Governor Calvo on June 15.

This grant proposal is a request for funds to hire a post doctoral entomologist for 2 years to work on an existing, funded project to implement effective biological control for the coconut rhinoceros beetle (CRB) which is rapidly killing palm trees in Guam and Palau. Without significant population suppression of CRB in Guam and Palau, it is likely that Guam and Palau will lose most of their palms and it is just a matter of time before other Micronesian Islands are invaded by CRB. There is a consensus among Pacific-based entomologists that biological control is the best, and possibly the only way, to stop palms from being killed on an island-wide basis. In addition, there is an urgency to suppress CRB populations in Guam and Palau to reduce the risk of accidental transport to other islands in Micronesia and beyond.

Yours sincerely,

Aubrey Moore

Aubrey Moore, PhD

D. Letters of Support

D.1. Regional Invasive Species Council

Micronesia Regional Invasive Species Council

Tamdad Sulog
Chairman
Regional Invasive Species Council
P.O. Box 463
Colonia Yap FSM 96943

July 06, 2017

Nikolao Pula, Director
Office of Insular Affairs
U.S. Department of the Interior
1849 C Street, N.W. Mail-Stop 2429
Washington, D.C. 20240

Dear Director Pula,

As Chair of the Regional Invasive Species Council for the Western Pacific, I am pleased to submit a letter of support for Dr. Moore's grant proposal to support his work towards biological control of coconut rhinoceros beetle (CRB) on Guam and Palau.

Without a reducing the high populations of CRB on these islands, it is just a matter of time before this pest spreads within Micronesia. If it gets onto the smaller islands and atolls where coconut is the "tree of life" this will be a humanitarian tragedy.

RISC has been asked, via a communique from the recent Micronesian Forum, which is a meeting of all the Micronesian Governors, to help find a solution to the rhino beetle problem. Dr. Moore is offering a promising solution, and I urge your office to fund his proposal.

Yours sincerely,

Tamdad Sulog,
Chief, Yap Agriculture and Forestry

D.2. Republic of Palau

To be sent separately.

E. Administrative Forms

E.1. SF-424

OMB Number: 4040-0002
Expiration Date: 01/31/2019

APPLICATION FOR FEDERAL ASSISTANCE SF-424 - MANDATORY

1.a. Type of Submission:
- [X] Application
- [ ] Plan
- [ ] Funding Request
- [ ] Other

Other (specify):

1.b. Frequency:
- [X] Annual
- [ ] Quarterly
- [ ] Other

Other (specify):

1.c. Consolidated Application/Plan/Funding Request?
Yes [ ] No [X] Explanation

1.d. Version:
- [X] Initial
- [ ] Resubmission
- [ ] Revision
- [ ] Update

2. Date Received: 7/7/2017

STATE USE ONLY:

3. Applicant Identifier:

5. Date Received by State:

4a. Federal Entity Identifier:

6. State Application Identifier:

4b. Federal Award Identifier:

7. APPLICANT INFORMATION:

a. Legal Name: College of Natural and Applied Sciences, University of Guam

b. Employer/Taxpayer Identification Number (EIN/TIN): 98-0032933

c. Organizational DUNS: 779908151

d. Address:

Street1: 303 Campus Drive

Street2:

City: Mangilao

County / Parish:

State: GU: Guam

Province:

Country: USA: UNITED STATES

Zip / Postal Code: 96923

e. Organizational Unit:

Department Name:

Division Name:

f. Name and contact information of person to be contacted on matters involving this submission:

Prefix: Dr.

First Name: Aubrey

Middle Name:

Last Name: Moore

Suffix:

Title: Entomologist

Organizational Affiliation: College of Natural and Applied Sciences, University of Guam

Telephone Number: 1-671-686-5664

Fax Number:

Email: aubreymoore@guam.net

APPLICATION FOR FEDERAL ASSISTANCE SF-424 - MANDATORY

8a. TYPE OF APPLICANT:

H: Public/State Controlled Institution of Higher Education

Other (specify):

b. Additional Description:

9. Name of Federal Agency:

Department of the Interior

10. Catalog of Federal Domestic Assistance Number:

CFDA Title:

11. Descriptive Title of Applicant's Project:

Biological Control of Coconut Rhinoceros Beetle Biotype G in Micronesia

12. Areas Affected by Funding:

Guam, Republic of Palau

13. CONGRESSIONAL DISTRICTS OF:

a. Applicant: Guam

b. Program/Project:

Attach an additional list of Program/Project Congressional Districts if needed.

Add Attachment | Delete Attachment | View Attachment

14. FUNDING PERIOD:

a. Start Date: Sep 1, 2017

b. End Date: Oct 31, 2019

15. ESTIMATED FUNDING:

a. Federal ($): $176,553

b. Match ($): $0

16. IS SUBMISSION SUBJECT TO REVIEW BY STATE UNDER EXECUTIVE ORDER 12372 PROCESS?

- [ ] a. This submission was made available to the State under the Executive Order 12372 Process for review on:
- [ ] b. Program is subject to E.O. 12372 but has not been selected by State for review.
- [x] c. Program is not covered by E.O. 12372.

APPLICATION FOR FEDERAL ASSISTANCE SF-424 - MANDATORY

17. Is The Applicant Delinquent On Any Federal Debt?

Yes ☐ No ☒ Explanation

18. By signing this application, I certify (1) to the statements contained in the list of certifications and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances** and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 218, Section 1001)**

** I Agree ☐

** This list of certifications and assurances, or an internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

Authorized Representative:

Prefix:	First Name:
Dr.	Lee

Middle Name:

Last Name: Yudin

Suffix:	Title:
	Dean / Director

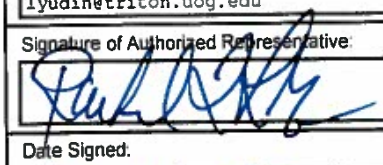
Organizational Affiliation: College of Natural and Applied Sciences, University of Guam

Telephone Number: (671) 735-2001

Fax Number:

Email: lyudin@triton.uog.edu

Signature of Authorized Representative:



Date Signed: July 6, 2017

Attach supporting documents as specified in agency instructions.

Add Attachments | Delete Attachments | View Attachments

E.2. SF-424A

BUDGET INFORMATION - Non-Construction Programs

OMB Number: 4040-0006
Expiration Date: 01/31/2019

SECTION A - BUDGET SUMMARY

Grant Program Function or Activity (a)	Catalog of Federal Domestic Assistance Number (b)	Estimated Unobligated Funds		New or Revised Budget		
		Federal (c)	Non-Federal (d)	Federal (e)	Non-Federal (f)	Total (g)
1. Biological control of coconut rhinoceros beetle	10.025	$	$	$ 176,553	$ 0	$ 176553
2.						
3.						
4.						
5. Totals		$	$	$	$	$

Standard Form 424A (Rev. 7- 97)
Prescribed by OMB (Circular A -102) Page 1

SECTION B - BUDGET CATEGORIES

6. Object Class Categories	GRANT PROGRAM, FUNCTION OR ACTIVITY (1)	(2)	(3)	(4)	Total (5)
a. Personnel	$ 120000	$	$	$	$
b. Fringe Benefits	21600				
c. Travel	17298				
d. Equipment	0				
e. Supplies	0				
f. Contractual	0				
g. Construction	0				
h. Other	17655				
i. Total Direct Charges (sum of 6a-6h)					$
j. Indirect Charges	0				$
k. TOTALS (sum of 6i and 6j)	$	$	$	$	$
7. Program Income	$ 0	$	$	$	$

SECTION C - NON-FEDERAL RESOURCES

(a) Grant Program	(b) Applicant	(c) State	(d) Other Sources	(e)TOTALS
8. Biological control of coconut rhinoceros beetle	$ 0	$	$	$
9.				
10.				
11.				
12. TOTAL (sum of lines 8-11)	$	$	$	$

SECTION D - FORECASTED CASH NEEDS

	Total for 1st Year	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
13. Federal	$	$ 0	$ 0	$ 0	$ 35455
14. Non-Federal	$	0	0	0	0
15. TOTAL (sum of lines 13 and 14)	$	$	$	$	$

SECTION E - BUDGET ESTIMATES OF FEDERAL FUNDS NEEDED FOR BALANCE OF THE PROJECT

(a) Grant Program	FUTURE FUNDING PERIODS (YEARS) (b)First	(c) Second	(d) Third	(e) Fourth
16. 2018	$ 23998	$ 17700	$ 17700	$ 17700
17. 2019	17700	17700	17700	10900
18.				
19.				
20. TOTAL (sum of lines 16 - 19)	$	$	$	$

SECTION F - OTHER BUDGET INFORMATION

21. Direct Charges: 176553

22. Indirect Charges: 0

23. Remarks:

E.3. SF-424B

OMB Number: 4040-0007
Expiration Date: 01/31/2019

ASSURANCES - NON-CONSTRUCTION PROGRAMS

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE OFFICE OF MANAGEMENT AND BUDGET. SEND IT TO THE ADDRESS PROVIDED BY THE SPONSORING AGENCY.

NOTE: Certain of these assurances may not be applicable to your project or program. If you have questions, please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

As the duly authorized representative of the applicant, I certify that the applicant:

1. Has the legal authority to apply for Federal assistance and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project cost) to ensure proper planning, management and completion of the project described in this application.

2. Will give the awarding agency, the Comptroller General of the United States and, if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related to the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.

3. Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.

4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.

5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. §§4728-4763) relating to prescribed standards for merit systems for programs funded under one of the 19 statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).

6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C.§§1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation Act of 1973, as amended (29 U.S.C. §794), which prohibits discrimination on the basis of handicaps; (d) the Age Discrimination Act of 1975, as amended (42 U. S.C. §§6101-6107), which prohibits discrimination on the basis of age; (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-255), as amended, relating to nondiscrimination on the basis of drug abuse; (f) the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism; (g) §§523 and 527 of the Public Health Service Act of 1912 (42 U.S.C. §§290 dd-3 and 290 ee-3), as amended, relating to confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. §§3601 et seq.), as amended, relating to nondiscrimination in the sale, rental or financing of housing; (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and, (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.

7. Will comply, or has already complied, with the requirements of Titles II and III of the Uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally-assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.

8. Will comply, as applicable, with provisions of the Hatch Act (5 U.S.C. §§1501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

9. Will comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. §§276a to 276a-7), the Copeland Act (40 U.S.C. §276c and 18 U.S.C. §874), and the Contract Work Hours and Safety Standards Act (40 U.S.C. §§327-333), regarding labor standards for federally-assisted construction subagreements.

10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of the Flood Disaster Protection Act of 1973 (P.L. 93-234) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is $10,000 or more.

11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11988; (e) assurance of project consistency with the approved State management program developed under the Coastal Zone Management Act of 1972 (16 U.S.C. §§1451 et seq.); (f) conformity of Federal actions to State (Clean Air) Implementation Plans under Section 176(c) of the Clean Air Act of 1955, as amended (42 U.S.C. §§7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended (P.L. 93-523); and, (h) protection of endangered species under the Endangered Species Act of 1973, as amended (P.L. 93-205).

12. Will comply with the Wild and Scenic Rivers Act of 1968 (16 U.S.C. §§1271 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.

13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. §470), EO 11593 (identification and protection of historic properties), and the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. §§469a-1 et seq.).

14. Will comply with P.L. 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.

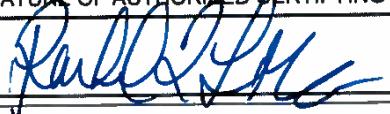
15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 89-544, as amended, 7 U.S.C. §§2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.

16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. §§4801 et seq.) which prohibits the use of lead-based paint in construction or rehabilitation of residence structures.

17. Will cause to be performed the required financial and compliance audits in accordance with the Single Audit Act Amendments of 1996 and OMB Circular No. A-133, "Audits of States, Local Governments, and Non-Profit Organizations."

18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations, and policies governing this program.

19. Will comply with the requirements of Section 106(g) of the Trafficking Victims Protection Act (TVPA) of 2000, as amended (22 U.S.C. 7104) which prohibits grant award recipients or a sub-recipient from (1) Engaging in severe forms of trafficking in persons during the period of time that the award is in effect (2) Procuring a commercial sex act during the period of time that the award is in effect or (3) Using forced labor in the performance of the award or subawards under the award.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL	TITLE
	Dean / Director
APPLICANT ORGANIZATION	DATE SUBMITTED

6 Appendix C: Post-doctoral Entomologist Position Announcement

See following page.

RESEARCH CORPORATION
OF THE UNIVERSITY OF GUAM

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ANNOUNCEMENT

THE RESEARCH CORPORATION OF THE UNIVERSITY OF GUAM SOLICITS APPLICATIONS TO ESTABLISH A LIST OF ELIGIBLES FOR THE FOLLOWING LIMITED TERM APPOINTMENT, 100% FEDERALLY FUNDED FULL-TIME POSITION (SUBJECT TO THE AVAILABILITY OF FUNDS):

Position Title
Post-Doctoral Researcher (Insect Pathologist) **JOB # RC-18-06**

***Application Deadline:* Until position is filled**
Send RCUOG application, transcripts, curriculum vitae, and one-page personal statement that outlines pertinent experience, general qualifications, graduate degrees and research fields to rcuoghr@triton.uog.edu. The RCUOG application can be downloaded from www.uog.edu/rcuog; located under the Forms subhead.

Salary
Grade O, Step1, $23.99 per hour
Full-time, 40 hours per week
Benefits: Medical and Dental Insurance, 4 hours annual/4 hours sick leave per pay period, holiday pay, Social Security and Medicare

The position begins upon **Selection Notification** ends on **September 30, 2019** based on availability of funds with possibility of extension should additional funds become available.

Location:
College of Natural and Applied Sciences at the University of Guam, Mangilao, Guam

MINIMUM QUALIFICATIONS:

- Ph.D. from an accredited college or university in Entomology, Zoology, or a related field
- Must be a U.S. citizen or a permanent resident.

MINIMUM KNOWLEDGE, ABILITIES, AND SKILLS:

- Experience in insect pathology and insect rearing

- Experience in performing bioassays to evaluate insect pathogens as potential biological control agents
- Ability to conduct field work under sometimes difficult field conditions (e.g. high temperature and humidity)
- Knowledge of the principals and practice of biological control.
- Ability to create and implement project design
- Ability to collect, organize, and analyze data
- Experience in report writing
- Supervisory experience
- Must possess a valid driver's license and a valid passport.

CHARACTER OF DUTIES:

The post-doctoral researcher will work with a project PI and collaborators to develop effective biological control for the coconut rhinoceros beetle Guam biotype (CRB-G). This recently discovered biotype has recently invaded several Pacific island groups were it is causing damage and mortality of coconut and oil palms. In the past, coconut rhinoceros beetle invasions were successfully controlled by introduction of Oryctes rhinoceros nudivirus (OrNV) as a classical biological control agent. However, CRB-G is resistant to all currently available isolates of OrNV.

The selected applicant will:

- Participate in foreign exploration for isolates of OrNV which are pathogenic to CRB-G. Foreign travel will be required.
- Perform bioassays to evaluate OrNV isolates as biocontrol agents for CRB-G
- Propagate promising OrNV isolates for autodissemination
- Author and co-author reports and peer-reviewed publications documenting research results

CLEARANCES

- College transcripts should be submitted with application
- Upon selection, the applicant must submit PPD, police and court clearances to UOG HRO.

7 Technical Report: Bioassay of OrNV Isolated from CRB-G, Negros Island, Philippines

See following page.

Dumaguete OrNV bioassay 1

January 11, 2018

1 Dumaguete OrNV Isolate - Bioassay 1

1.1 Introduction

This notebook documents an initial bioassay of the Dumaguete isolate of *Oryctes* nudivirus.

1.2 Materials and Methods

1.2.1 Virus Sample

This virus was isolated from a single infected *Oryctes rhinoceros* Guam biotype specimen collected near Dumaguete, Negros Island, Philippines in January 2017.

1.2.2 Test Insects

Test insects were collected weekly from 31 coconut rhinoceros beetle pheromone traps (barrel traps) at the University of Guam Agricultural Experiment Station at Yigo. Beetles were held individually in numbered Mason jars partially filled with moist peat moss which were stored in an environmental cabinet at a temperature of 80 degrees F. Each was fed a slice of banana weekly. Beetles were reared an average of 46 days (range: 23 d to 75 d) prior to treatment.

1.2.3 Treatment

- 20 beetles were selected at random for treatment and another 20 were selected for experimental control
- 100 mg of sucrose was disolved in a 1 ml sample of the virus. Each beetle was given a 40 microlitre dose of this solution by pipetting a drop onto its mouthparts. A placebo was not given to beetles in the experimental control group.
- All beetles were provided a slice of banana on the day following treatment.

1.2.4 Observation procedure

- Beetles were observed weekly.
- Each beetle was weighed and its mass was entered immediately into a spreadsheet.
- A slice of banana was added immediately after each beetle was returned to its jar.

1.2.5 Analysis

- The data model includes 2 tables saved as Excel spreadsheets (see below).
- An empty **mass** field in the **observations** table indicates that the beetle died prior to observation.

1.3 Results and Discussion

- The beetles did not readily drink the 40 microlitre dose applied to their mouthparts. A food incorporation bioassay may be more efficient.
- During the 28 day bioassay period, 1 of 20 virus treated beetles died and 4 of 20 beetles in the experimental control group died. The difference in mortality is not significant (p = 0.34; Fisher's exact test). Post mortems indicated that all of the dead beetles were infected with *Metarhizium majus*. Guts looked normal.
- There was a significant difference in weight loss during the first week of the bioassay (p = 0.02; Welch's t-test). However, there was no significant difference in weight loss during succeding weeks. Difference in weight loss during the first week may be due to the fact that the virus treated beetles were handled much more than the contol group.
- In conclusion, results from this initial bioassay does not indicate pathogenicity to CRB-G for the OrNV Dumaguete isolate.

1.4 Calculations

```
In [22]: import pandas as pd
         import matplotlib.pyplot as plt
         import sqlite3
         import numpy as np
         from scipy.stats import ttest_ind, fisher_exact

         %matplotlib inline
         pd.options.display.max_rows = 4
```

1.4.1 import data from spreadsheet

```
In [23]: DATAFILE = 'aubrey.xlsx'

         df_jars = pd.read_excel(DATAFILE, 'jars')
         df_jars
```

```
Out[23]:     jar_number date_collected     sex treatment
         0           32     2017-10-02  Female     virus
         1           85     2017-10-02  Female     virus
         ..         ...            ...     ...       ...
         38        1263     2017-10-18    Male   control
         39        1633     2017-10-23    Male   control

         [40 rows x 4 columns]
```

```
In [24]: df_observations = pd.read_excel(DATAFILE, 'observations')
         df_observations
```

Out[24]:

	jar_number	observation_date	mass	note
0	32	2017-11-15	4487.0	NaN
1	50	2017-11-15	4136.0	mites
..	...	...	...	...
198	1263	2017-12-13	3036.0	NaN
199	1633	2017-12-13	3399.0	NaN

[200 rows x 4 columns]

In [25]:
```
# Create a dict containing pairs of dates.
# The index is an observation date and the value is the previous observation date

obs_dates = df_observations.observation_date.unique()
prev_obs_date = dict(zip(obs_dates[1:], obs_dates))

# Example usage:
# y = df_observations.observation_date[100]
# print y
# prev_obs_date[y.to_datetime64()]
```

In [26]:
```
# Add a new column "days_post_treatment" to "df_observations" and populate it

treatment_date = df_observations.observation_date.min().to_datetime64() # Assumes all t
df_observations['days_post_treatment'] = np.nan

for index, row in df_observations.iterrows():
    try:
        date = row.observation_date.to_datetime64()
        if date == treatment_date:
            days_post_treatment = 0
        else:
            days_post_treatment = date - treatment_date
            # Convert from timedelta in nanoseconds to integer days
            days_post_treatment = (days_post_treatment / np.timedelta64(1, 'D')).astype
    except:
        continue
    df_observations.loc[index, 'days_post_treatment'] = days_post_treatment

# Add a new column "mass_change" to "df_observations" and populate it

df_observations['mass_change'] = np.nan

for index, row in df_observations.iterrows():
    try:
        date = row.observation_date.to_datetime64()
        prev_date = prev_obs_date[date]
        prev_mass = int(df_observations.loc[(df_observations.observation_date==prev_dat
            (df_observations.jar_number==row.jar_number), 'mass'])
```

```
            mass_change = int(row.mass) - prev_mass
        except:
            continue
        df_observations.loc[index, 'mass_change'] = mass_change

df_observations
```

Out[26]:

	jar_number	observation_date	mass	note	days_post_treatment	mass_change
0	32	2017-11-15	4487.0	NaN	0.0	NaN
1	50	2017-11-15	4136.0	mites	0.0	NaN
..	...	...	...	...	...	...
198	1263	2017-12-13	3036.0	NaN	28.0	258.0
199	1633	2017-12-13	3399.0	NaN	28.0	-194.0

[200 rows x 6 columns]

In [27]:
```
df = df_observations.merge(df_jars, on='jar_number')
df
```

Out[27]:

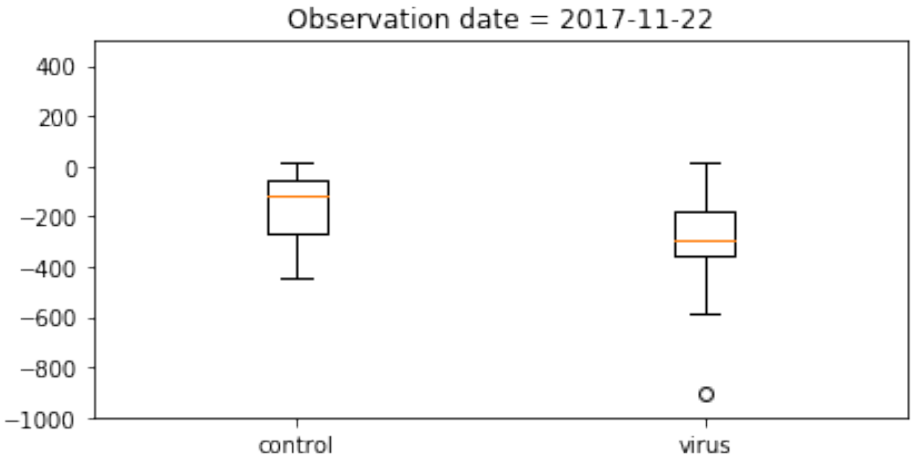
	jar_number	observation_date	mass	note	days_post_treatment	mass_change	date_collected	sex	treatment
0	32	2017-11-15	4487.0	NaN	0.0	NaN	2017-10-02	Female	virus
1	32	2017-11-22	3900.0	NaN	7.0	-587.0	2017-10-02	Female	virus
..	...	...	...	...	...	...	...	...	...
198	1633	2017-12-06	3593.0	NaN	21.0	447.0	2017-10-23	Male	control
199	1633	2017-12-13	3399.0	NaN	28.0	-194.0	2017-10-23	Male	control

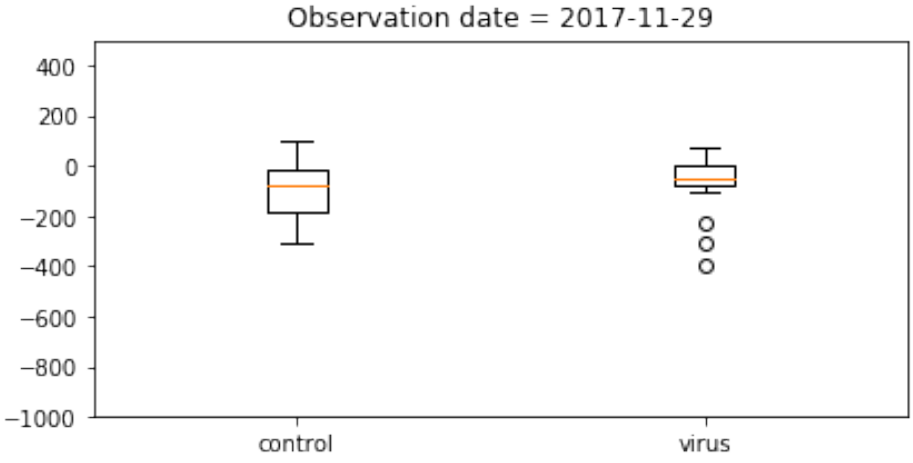
[200 rows x 9 columns]

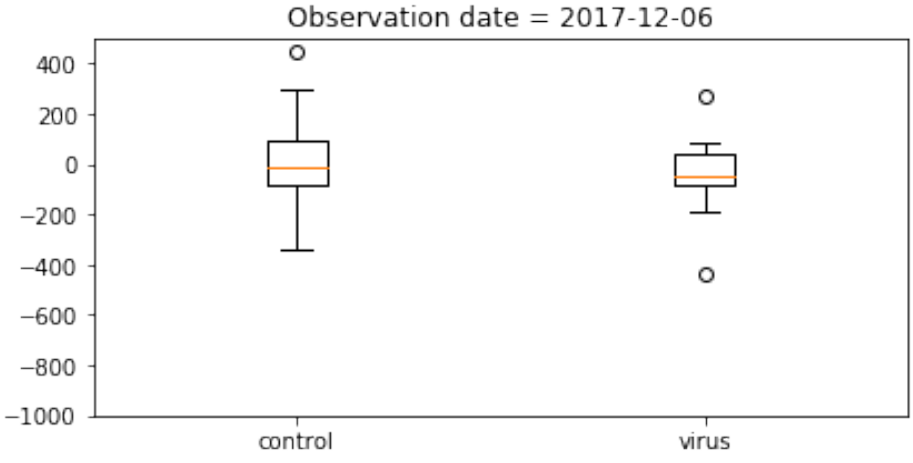
In [28]:
```
fig = plt.figure(figsize=(6, 12))
i = 0
print 'observation \tcontrol \t\tvirus'
print 'date \t\tmean mass change (mg) \tmean mass change (mg) \tp-value'
for date in prev_obs_date.keys():
    i += 1
    v = df.loc[(df.observation_date==date) &
```

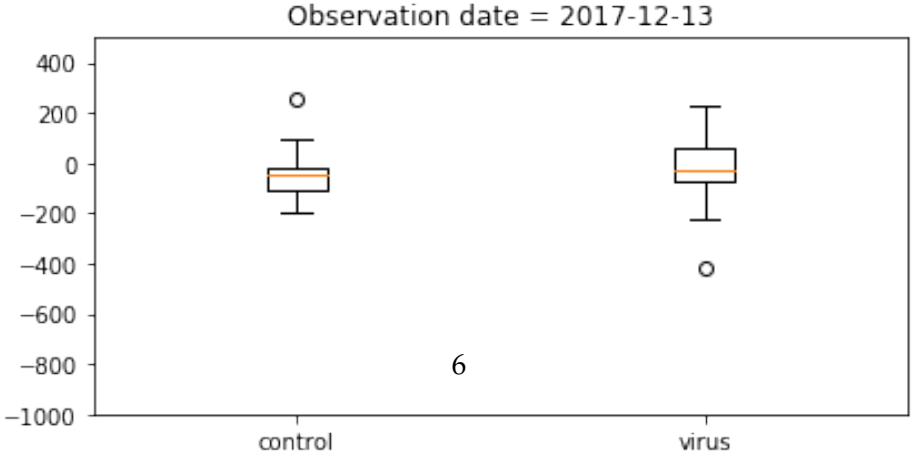
```
                                (df.treatment=='virus'), 'mass_change']
            v = v[-np.isnan(v)]
            c = df.loc[(df.observation_date==date) &
                                (df.treatment=='control'), 'mass_change']
            c = c[-np.isnan(c)]

            statistic, pvalue = ttest_ind(c, v, nan_policy='omit', equal_var=False)
            print '{}\t{:.0f} (n={})\t\t{:.0f} (n={})\t\t{:.4f}'.format(
                str(date)[:10], np.mean(c), len(c), np.mean(v), len(v), pvalue)
            ax = fig.add_subplot(4,1,i)
            title = 'Observation date = ' + str(date)[:10]
            ax.set_title(title)
            ax.boxplot([c.values, v.values])
            ax.set_xticklabels(['control', 'virus'])
            ax.set_ylim([-1000, 500])
        plt.tight_layout()
```

```
observation         control                 virus
date                 mean mass change (mg)         mean mass change (mg)         p-value
2017-11-22        -163 (n=20)                -298 (n=20)                0.0195
2017-11-29        -107 (n=19)                -70 (n=20)                0.3216
2017-12-06        3 (n=18)                -40 (n=20)                0.4429
2017-12-13        -41 (n=16)                -37 (n=19)                0.9350
```


Observation date = 2017-11-22
400
200
0
−200
−400
−600
−800
−1000
control
virus


Observation date = 2017-11-29
400
200
0
−200
−400
−600
−800
−1000
control
virus


Observation date = 2017-12-06
400
200
0
−200
−400
−600
−800
−1000
control
virus

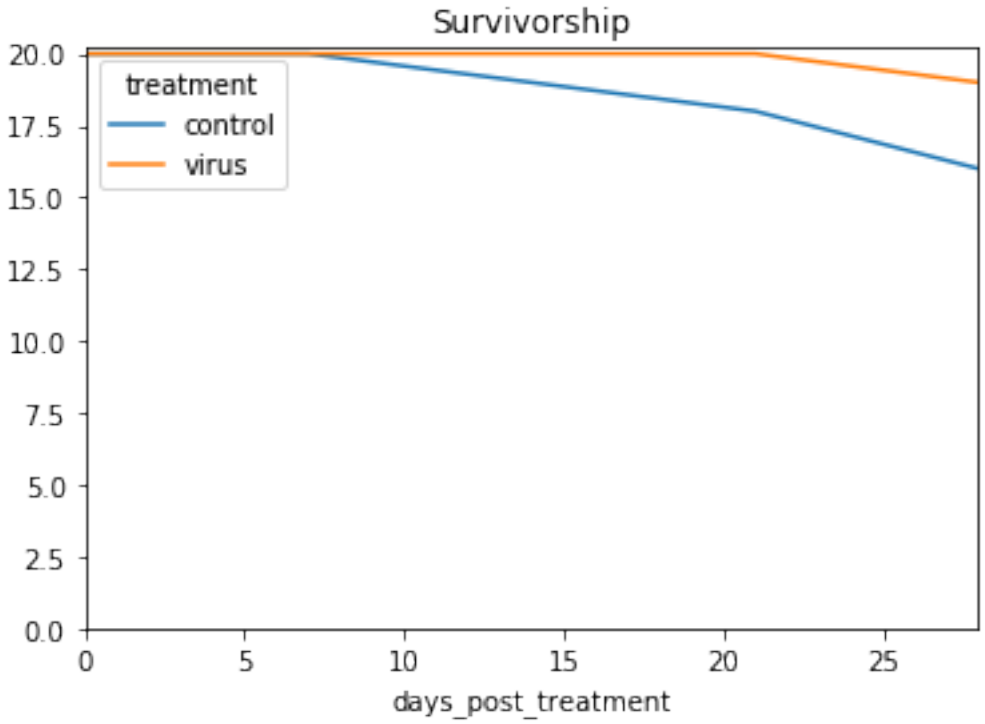

Observation date = 2017-12-13
400
200
0
−200
−400
−600
−800
−1000
control
virus

1.4.2 Mortality

In [29]:
```
df_groupby = df.groupby(['days_post_treatment','treatment'])['mass'].count().unstack()
df_groupby
```

Out[29]:
```
treatment             control  virus
days_post_treatment
0.0                        20     20
7.0                        20     20
...                       ...    ...
21.0                       18     20
28.0                       16     19

[5 rows x 2 columns]
```

In [34]:
```
myplot = df_groupby.plot()
ylim = myplot.axes.get_ylim()
myplot.axes.set_ylim(0, ylim[1])
myplot.set_title('Survivorship');
```



In [31]:
```
c_total = df_groupby.control.max()
c_alive = df_groupby.control.min()
```

```
        c_dead = c_total - c_alive
        v_total = df_groupby.virus.max()
        v_alive = df_groupby.virus.min()
        v_dead = v_total - v_alive
        print '{}\t{}\t{}'.format('', 'alive', 'dead')
        print '{}\t{}\t{}'.format('control', c_alive, c_dead)
        print '{}\t{}\t{}'.format('virus', v_alive, v_dead)
        print
        oddsratio, pvalue = fisher_exact([[c_alive, c_dead], [v_alive, v_dead]])
        print 'Fisher''s exact test p-value = {}'.format(pvalue)
```

```
        alive   dead
control 16      4
virus   19      1

Fishers exact test p-value = 0.341649341649
```

1.4.3 Holding time prior to treatment

```
In [32]: # Calculate days held prior to treatment

        pd.options.display.max_rows=100
        df_temp = df[df.observation_date==treatment_date]
        (df_temp.observation_date - df_temp.date_collected).describe()
```

```
Out[32]: count                         40
         mean            46 days 15:00:00
         std      18 days 08:07:05.397423
         min             23 days 00:00:00
         25%             33 days 00:00:00
         50%             44 days 00:00:00
         75%             57 days 00:00:00
         max             75 days 00:00:00
         dtype: object
```