USDA-Forest Service Project Proposal

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1 Project Number

15-DG-11052021-205

2 Project Title

Detector Beetles: Radio-Tracking Coconut Rhinoceros Beetles (CRB) to Discover Breeding Sites and CRB Biocontrol

3 Fiscal Year of Project Submittal

FY2015

4 Expected Completion Date

30 April, 2016

5 Status of Subject Species

non-native invasive

6 Brief Description of Project

This proposal has been revised in response to an opportunity for a funding increase from $20K to $40K. The original plan was to test the feasibility of using 'detector beetles' as a rapid response/eradication tactic for finding CRB breeding sites. This tactic will have little utility on Guam, where the eradication attempt has failed, but it may be critical for increasing probability of eradication on Oahu and other new invasions.

The additional $20K will be used as seed money to establish a project with the objective of finding a strain of Oryctes nudivirus (OrNV) which is highly pathogenic for the newly discovered Guam biotype of CRB. The Guam CRB biotype, currently found on Guam, Oahu, and Palau (see APHIS biocontrol project report), is resistant to OrNV and is likely to spread to other islands. Prior to emergence of the Guam CRB biotype, OrNV was used as a very effective classical biocontrol agent to mitigate damage whenever Pacific islands were invaded by CRB (**Bedford2013**). Given that CRB eradication for this species is very difficult, achieved on only one island out of many where it has been attempted, it is very important to have a density-dependent biocontrol agent available for cases in which eradication fails (Guam) or an eradication attempt is not feasible. Despite significant progress in development of effective IPM tactics for CRB, these are not sufficient to limit CRB damage to acceptable levels without an effective density-dependent biocontrol component.

6.1 Detector Beetles

Eradication and local extirpation of the coconut rhinoceros beetle (CRB) requires location and destruction of breeding sites where all life stages aggregate. Adult males and females find these sites during flight by detecting an aggregation pheromone and possibly other chemical and physical stimuli.

We propose a field trial on Guam to test the feasibility of locating CRB breeding sites by radio-tracking adults with miniature radio transmitters attached to them. Members of the research team have experience in radio-tracking insects and this technology is likely to work very well with this large beetle. The field trial will be performed on Guam during a 7 to 10 day period during FY2015. Additional funding for further research will be requested if the feasibility test is a success.

6.2 Oryctes Nudivirus as a Biocontrol Agent for the Guam Biotype of CRB

In collaboration with Sean Marshall, AgResearch New Zealand, we have determined that the rhino beetle attacking Guam's palms is genetically distinct that those elsewhere in the Pacific, except for those invading Hawaii and part of the CRB population in Palau, and that this new biotype is apparently resistant to OrNV, which has been used as a very effective density-dependent biocontrol agent for CRB wherever it has invaded Pacific Islands.

Bioassays of several isolates of Oryctes nudivirus provided by AgriResearch New Zealand failed to result in significant pathogenicity for the Guam CRB genotype. In a 'last ditch' attempt we made a 'witches brew' slurry containing all frozen dead beetles from previous bioassays plus frozen virus samples in vials. Forty adult beetles were forced to swim in

the slurry for 30 minutes on January 22, 2015. A control group of 41 beetles were forced to swim in water. Beetles were checked weekly.

By May 10, 2015, mortality of the virus treated beetles (78%) was significantly greater than that of the control group (54%).

This experiment is incomplete. A postmortem will be done on the dead beetles and the 'witches brew' process needs to be repeated to see if this also results in significant mortality.

Project funds will be used to hire Ian Iriarte, who did the original work, as a graduate assistant to complete the 'witches' brew bioassay. He will also help establish a biocontrol lab to facilitate discovery of an OrNV strain which is highly pathenogenic for the CRB-Guam biotype.

Although invasion by CRB-Guam is a regional problem, it is anticipated that Guam will be the location at which OrNV candidates from foreign exploration will be tested. The project PI already has USDA-APHIS permits to import OrNV and CRB into Guam. The graduate student will assist in establishing an insect pathology lab at the University of Guam to be used for applied research in CRB biocontrol.

7 Project Objectives

7.1 Detector Beetles

- to develop radio tracking techniques to follow adult CRB in the field
- to find at least one undiscovered CRB breeding site

7.2 OrNV for CRB-Guam

- to find of an OrNV strain which is highly pathenogenic for the CRB-Guam biotype
- establishment of an insect pathology lab at the University of Guam to be used for applied research in CRB biocontrol

8 Justification and Urgency/Product Leveraging

8.1 Detector Beetles

- The detector beetle concept has potential as a rapid response tactic in a new CRB infestation. If only male detector beetles are used, this will not significantly increase fecundity and they will cause only minor, short-term damage.
- Radio-tracking beetles to find cryptic breeding sites may prove to be a critical tactic for eradication of CRB on Oahu
- The technique is scalable and can be automated: rapid surveys of large areas can be done by releasing large numbers of radio-tagged adult males and then tracking these using an aircraft based data-logging receiver or automated terrestrial receiver grid

- The technique is a very cost effective alternative to the use of detector dogs. The beetles require no training and care and maintenance costs are negligible.
- There are precedents for using one insect to find another at low population densities (e.g. philanthine wasps, *Cerceris fumipennis*, used to detect buprestid beetles, especially the emerald ash borer (**swink2013**).

8.2 OrNV for CRB-Guam

The need to find a strain of OrNV or other density-dependent biocontrol agent for the CRB-Guam biotype is urgent. In a worst-case scenario a population explosion of CRB on Guam could result in high palm mortality. The resulting dead palms would provide abundant CRB breeding sites, creating a positive feedback loop where even more CRB adults are generated, killing even more palms. An effective density-dependent biocontrol agent for CRB-Guam, such as a highly pathogenic OrNV strain, is needed to counteract this feedback process.

A trigger for CRB population outbreaks is abundant green waste left in the wake of typhoons. Typhoon Dolphin past over Guam in May 2015, leaving much decaying vegetation behind. The Guam CRB population is expected to increase during 2015, increasing the risk of accidental export of this pest to other islands.

9 Scope of Application

9.1 Detector Beetles

The scope of this project is limited to radio-tracking adult coconut rhinoceros beetles in order to find aggregations at breeding sites. However, similar techniques could be developed for any large pest insects.

9.2 OrNV for CRB-Guam

If we are successful in finding a strain of OrNV which is highly pathogenic for CRB-Guam, this can be propogated for auto-dissemination using well known methods for *in vivo* or *in vitro* propagation.

10 Measures of Success

10.1 Detector Beetles

10.1.1 Expected Outcomes

We expect to demonstrate the feasibility of detecting cryptic CRB breeding sites by tracking radio-tagged beetles.

10.1.2 Products and Due Dates

A ten-day intensive field trial will be done will be done on Guam during the first half of 2015 to test the feasibility of the detector beetle concept. A final report will be prepared within three months after conclusion of the field trial.

10.1.3 Benefits

Rapid detection and destruction of CRB breeding sites is critical to the success of CRB control and eradication programs. Tracking radio-tagged beetles may prove to be a cost-effective method of detecting CRB breeding sites at very low population levels. This highly sensitive detection methodology is particularly needed in the late stages of eradication projects.

10.2 OrNV for CRB-Guam

10.2.1 Expected Outcomes

Completion of the 'witch's brew' experiment and microscopic examination of dead beetles will give us a definative answer as to whether or not any of OrNV from AgResearch previously tested are pathogenic for CRB-Guam.

I expect that foreign exploration will be required to map the origin and geographic range of CRB-Guam, and to find OrNV biocontrol agent candidates which are impacting these populations. If awarded, this grant will help set up an insect pathology lab on Guam where bioassays of candidate OrNV isolates can be evaluated.

10.2.2 Products and Due Dates

The 'witch's brew' experiment should be completed and reported before December 2015.

10.2.3 Benefits

An effective OrNV density-dependant biocontrol agent will prevent outbreaks of CRB-Guam, maintain damage at acceptable levels, and reduce accidental export of the pest to other islands.

11 Technology Transfer

11.1 Detector Beetles

After further development, it is hoped that CRB radio-tracking technology will be rapidly transferred to USDA APHIS and Hawaii Department of Agriculture staff working on the eradication project currently under way on the Island of Oahu, in and around Honolulu. All members of our proposed research team have served as subject matter experts for the Hawaii CRB Eradication Project and two of them (EJ and AH) live and work in Hawaii. Hawaii CRB Eradication Project managers and staff will be briefed and trained in the use of beetle radio tracking protocols.

Further refinement of tracking protocols, beyond the scope of this pilot study, will likely need to be undertaken to tailor this technology to the Hawaiian context. Radio-tracking equipment on Guam may be readily deployed following early detection of CRB on other islands in Micronesia.

11.2 OrNV for CRB-Guam

If effective OrNV strains are found, these will be shared freely with collaborators.

12 Research Basis

12.1 Detector Beetles

Radio tracking has been used successful with several large scarab beetles in tropical areas, namely Taiwan (**mccullough2012**) and Papua New Guinea (**beaudoin2003flight**). **mccullough2012** was able to track 17 individual beetles with tracking times ranging from 1 to 10 days. In this study beetles were never tracked moving more than a half a kilometer. By contrast, **beaudoin2003flight** tracked 15 individual beetles, many of which flew out of range ($>$ 1 km, mostly females) shortly after being released. However, males that were able to be tracked, were observed in both feeding and breeding sites in a variety of microhabitats.

One of us (MS) has been working with radio tracking a large moth, the fruit piercing moth, *Eudocima phalonia*, over the last two years. While this moth is smaller than the coconut rhinoceros beetle, it was easily able to carry the transmitter and performed well in a number of flight tests both in Hawaii and northern Australia. Moths with radio transmitters were released in northern Australia and followed over the course of several days through orchards, farmland, and open forested areas. This success with previous insect tracking gives us confidence that we can successfully track the coconut rhinoceros beetle in the field.

Insects have highly developed olfactory systems and search behaviors that allow them to find conspecifics, host and breeding sites, and/or prey. We are not the first to propose or develop techniques using one insect to find another. **swink2013** detailed the use of philanthine wasps, *Cerceris fumipennis*, to detect buprestid beetles, especially the emerald ash borer. Buprestid beetles can be can be difficult to find in the environment and especially so as a new invasive. Early detection of the emerald ash borer, with the help of these wasps, is an example of using an insect's behavior to help with delimitation of a low population density pest.

12.2 OrNV for CRB-Guam

Prior to discovery of the CRB-Guam biotype, OrNV was well-known as a very successful biocontrol agent for CRB, expecially on recently-invaded Pacific islands (CITATIONS).

13 Methods

13.1 Detector Beetles

- Male beetles will be measured, weighed and flight tested beetles prior to selection for tagging, using **VanderMeer1987** as a guide.
- A miniature radio transmitter (Advanced Telemetry Systems (ATS) 2414) will be glued to the thorax of each selected beetle (Figure 1). Each transmitter has a unique frequency and is detectable for at least 0.5 km with ATS R410 scanning receivers.
- Beetles with transmitters will be released shortly after sunset in large open areas (e.g. golf courses) and tracked immediately with two person teams. Each team

member equipped with a scanning radio receiver, a Yagi antenna, and a GPS receiver. Matt Siderhurst will bring two students, who are experienced radio-trackers, to Guam to assist in the field trial.

- Subsequent releases will test whether beetles can be found without continual following by tracking teams. The flight period for CRB is during three hours following sunset. This beetle does not fly during the day, so it should be possible to find the exact location of all detected radio tags during each day for several days after beetle release. We expect that some of the tagged beetles will be found at breeding sites.

- Beetle movements will be recorded as GPS waypoints and mapped using GIS software.

13.2 OrNV for CRB-Guam

In a previous experiment, Frozen, dead beetles from 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.

In response to finding significantly higher mortality in beetles which swam in the slurry as opposed to beetles which swam in water, we will do post-mortem observations of all dead beetles from this experiment to see if we can find any sign of virus-induced pathology. Bodies will then be made into a slurry with which healthy beetles will be challenged.

14 Appendix 1- Budget

14.1 Budget Request

	Item	Requested FHP STDP Funding	Other Source Funding	Source
FY 2015				
Administration	Salary	$16,890.00		
	Benefits	$1,292.09		
	Overhead	$1,818.21		
	Travel	$12,410.00		
Procurements	Contracting	$0		
	Equipment	$0		
	Supplies	$7,589.70		
Year Total		$40,000.00		

14.2 Budget Request Explanation

Salary: Graduate student assistant (Ian Iriarte) at $15.00 per hour for 1126 hours
Benefits: Social security and Medicare at 7.65% of salary.
Overhead: Indirect costs to UOG at 10% of salary plus benefits.
Travel: Roundtrip airfare and per diem for 10 days on Guam.

- Airfare for Matt Siderhurst and 2 students, VA-Guam: 3 * $1706.66 = $5120
- Per diem: 3 people * 10 d * $243 = $7290

Supplies: Radio-tracking equipment will be purchased from Advanced Telemetry Systems (ATS) to match the receivers and antennae owned by Aubrey Moore and Matt Siderhurst. All 4 tracking systems will be used in the field trial. GPS equipment will be borrowed from the Guam CRB Project.

- glue-on transmitters ATS A2414: 30 * $190 = $5,700
- Yagi antennas 162-166 MHz - ATS 13863: 2 * $120 = $240
- receivers - ATS R410: 2 * $825 = $1,650

15 Appendix 2 Cooperators

15.1 FHP Lead Contact

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16 Appendix 3 – Literature Citations, Figures, Tables, Attachments, etc.

Figure 1: Greyback cane beetle with a miniature radio transmitter and antenna glued to its thorax.