College of Tropical Agriculture and Human Resources
Department of Plant and Environmental Protection Sciences

UNIVERSITY of HAWAIʻI
MĀNOA

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FINAL REPORT

Project Title: Management of Coconut Rhinoceros Beetle in Hawaii

Project Period: November 1, 2017 to April 30, 2019

Sponsor: Hawaii Invasive Species Council

Funding Amount: $100,000

Project Directors: Michael Melzer, Zhiqiang Cheng, and Daniel Jenkins

Affiliation: University of Hawaii
College of Tropical Agriculture and Human Resources
Plant and Environmental Protection Sciences

Contact: Michael Melzer
(808) 956-7887
melzer@hawaii.edu

Executive Summary

A large multi-agency response is underway to eradicate coconut rhinoceros beetle (CRB) on Oahu. Few methods are available for CRB control, and the development of additional tools for both short- and long-term control is critical. The goal of this project is to develop these tools. We proposed 1) to maintain the CRB colony to ensure CRB are available to researchers in Hawaii and abroad; 2) develop and demonstrate the use of genetic pesticides (RNAi) against CRB larvae under laboratory conditions; and 3) engineer and deploy smart CRB traps with improved efficacy and integrated data collection. From the colony, over 2300 CRB specimens were distributed to colleagues for research and outreach purposes. This total does not include the thousands of CRB specimens used for research purposes by the Melzer Lab. We were able to identify and develop dsRNAs for 5 RNAi target genes that induced high mortality in all CRB life stages. These dsRNAs should be further explored for practical use in the field. Progress has been made on the development of smart traps, and an engineered trap was field-deployed during the project period, and demonstrated its ability to capture adult CRB from the environment.

Overall Project Progress

OBJECTIVE 1. MAINTAIN THE CRB COLONY AT THE UNIVERSITY OF HAWAII.
A containment laboratory for CRB was established at the University of Hawaii in the summer of 2016. Trap-caught CRB are quarantined for one week, then used as the source of the breeding population. Eggs from the breeding population are collected weekly and maintained in a locally-sourced compost mix through the larval, pupal, and adult life stages. These life stages are then used in experiments aimed at the detection or control of CRB by our group and colleagues.

The CRB colony has become more efficient due to rearing improvements that lead to higher female fecundity. As a result, the total number of adults kept for the rearing population is maintained at approximately 100 females and 50 males. During the project period of 11/1/17 through 4/30/19, 614 adult females and 571 adult males were reared from eggs collected in the colony.

Colony Metrics per 6 Month Period	**11/1/17 – 4/30/18**	**5/1/18 – 10/31/18**	**11/1/18 – 4/30/19**
Average Eggs/Week (Range)	447 (190-886)	738 (301-1177)	868 (515-1430)
Average Females in Colony	194	102	101
Average Fecundity (Range)	3 (0.7-5.5)	7 (3.9-11)	9 (5.5-12.2)

Colony-reared CRB used to populate detection and control experiments conducted by our collaborators (UH and USDA) from Sep 1, 2018 to Feb 28, 2019. This does <u>not</u> include CRB used in the Melzer program's research trials:

USDA-APHIS-CPHST Otis Lab in Buzzards Bay, MA during the project period:

Nov 2017 – 24 adults
Feb 2018 – 12 adults
May 2018 – 24 adults
Oct 2018 – 12 adults
Nov 2018 – 12 adults
Feb 2019 – 12 adults

CRB for Dr. Zhiqiang Cheng's research activities during the project period:

November 6, 2017 – 250 first instar larvae (fungal trial)
January 10, 2018 – 250 first instar larvae (fungal trial)
February 6, 2018 – 203 first instar larvae (fungal trial)
March 2018 – 200 first instar larvae (fungal trial)
April 18 2018 – 260 first instar larvae (fungal trial)
May 10, 2018 – 260 first instar larvae (fungal trial)
June 5 2018 – 260 first instar larvae (fungal trial)
September 5 2018 – 200 first instar larvae (fungal trial)
November 21 2018 – 200 first instar larvae (fungal trial)
Jan-Feb 2019 – 16 third instar larvae (fungal trial)
April 2019 – 32 adult beetles (chemical trial)
April 2019 – 4 first instar (nematode trial)

Preserved colony-reared CRB (all life stages) were distributed to collaborators for displays

California Department of Forestry and Fire Prevention (Feb 2019)

USDA-APHIS-PPQ Kahului International Airport (OGG) (Feb 2019)

USDA-ARS PBARC (Hilo) for research (March 2019)

20 eggs

20-1st instars

15-2nd instars

8-3rd instars

4-adults

Visitors and tours:

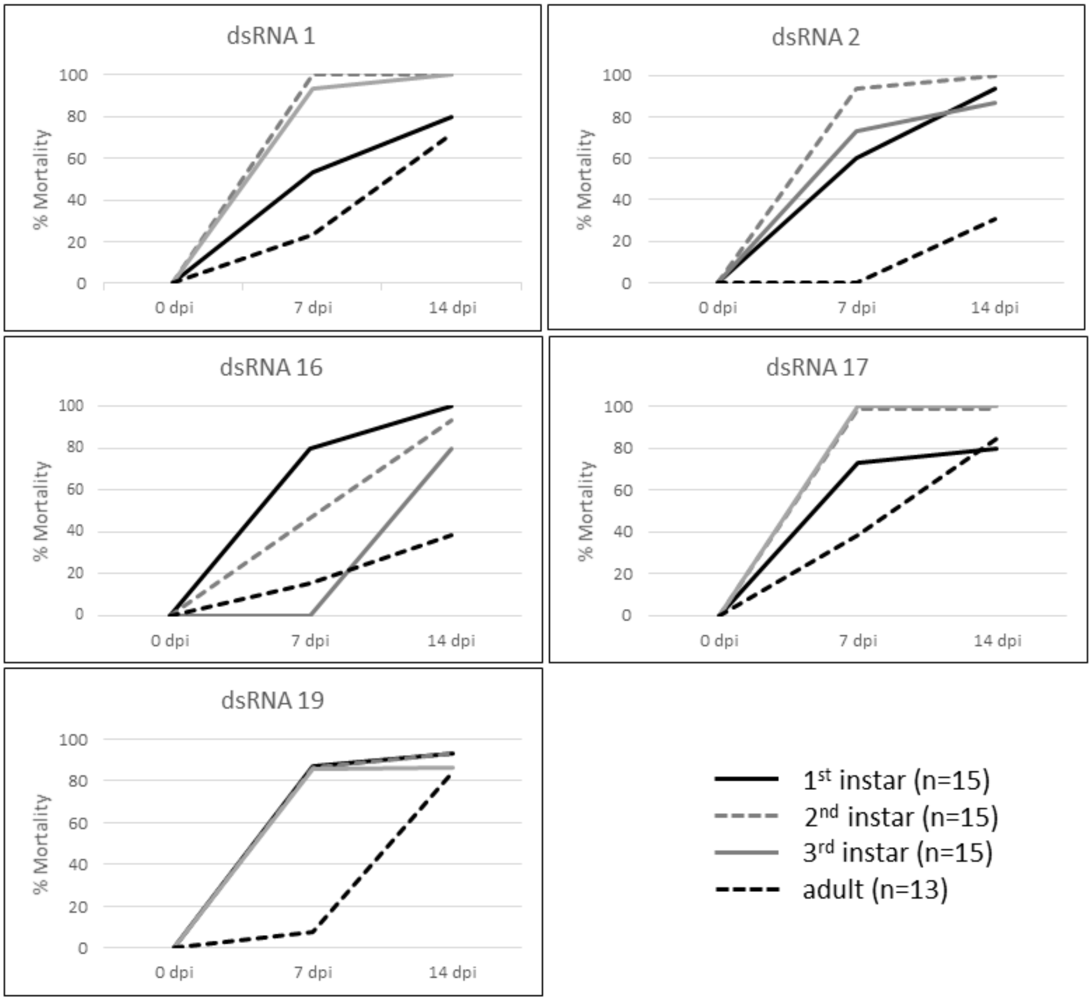
- 12/8/2017: Harry Kaya, Madoka Nakai, Janet Kabashima, John Kabashima
- 12/15/18: Community (Manley tour – 3 people)
- 12/22/18: Community (Manley tour – 3 people)
- 1/5/18: Abigail Anzelmo (U of WI)
- 1/24/18: CTAHR Fiscal office (3 people)
- 2/9/18: Community (Schlieman – 3 people)
- 2/27/18: Deron Smith (USDA), Sherrena Harrison, Anton Davis)
- 3/14/18: B. Cl? M. Ero (PNG OPRA)
- 4/4/18: Chris Kadooka (Photography)
- 4/23/18: Community/UH PreVet Club – Rebecca Smith, daughter & friend
- 6/27/18: Osama El-Lissy, Diana Hoffman, Matt Roger (USDA)
- 8/1/18: USDA Hawaii Port Officials (Tony Nakamura, Noel Hashimoto, Stuart Stein, Mike Scharf, Haroo Taira, Julie Yogi-Chun)
- 10/8/18: Community (9 members of the UH Pre-Vet Club)
- 12/6/19: Tour: L. Brevington (East-West Center), R. Quitugua (U. of Guam), P. Andreozzi (USDA)
- 12/11/19: UH Biosafety Inspection (H. Olipares)
- 2/13/19: Tour: E. Palko, P. Waisen (UH Colleagues)
- 3/28/19: Tour: D. Crook, R. Haff (Collaborators)
- 4/3/19: Tour: N. Hoffman (Honolulu Botanical Gardens)
- 4/4/19: Tour: CTAHR Fiscal office (5 people)
- 4/15/19: Pacific Islands Forestry Workshop (23 Forestry visitors from multiple Pacific Islands)
- 4/22/19: Student STEM Experiment (N. Harris)
- 4/26/19: Tour (M. Heideveen - Representative for Ed Case, K. Sewake)

OBJECTIVE 2. DEVELOP AND DEMONSTRATE THE USE OF GENETIC PESTICIDES AGAINST CRB. RNAi-based insecticides are an emerging class of pesticides that are environmentally friendly, highly host specific, and theoretically immune to the development of resistance. Typical targets for insects are housekeeping genes, and the active molecules are short (100-500 bp) double-stranded (ds)RNAs. RNAi has been successfully used against aphids, psyllids, and several lepidopterans that infest corn. There is a paucity of genetic information available for CRB, so we used high throughput sequencing to characterize the transcriptome of the gut tissue of early instar CRB specimens. From these data we identified and performed a preliminary evaluation of 23 target genes using 2nd instar larvae and adults. The dsRNA targeting five of these genes induced mortality: 3 actin genes and the genes encoding a vacuolar type ATPase and a polyadenylate binding protein:

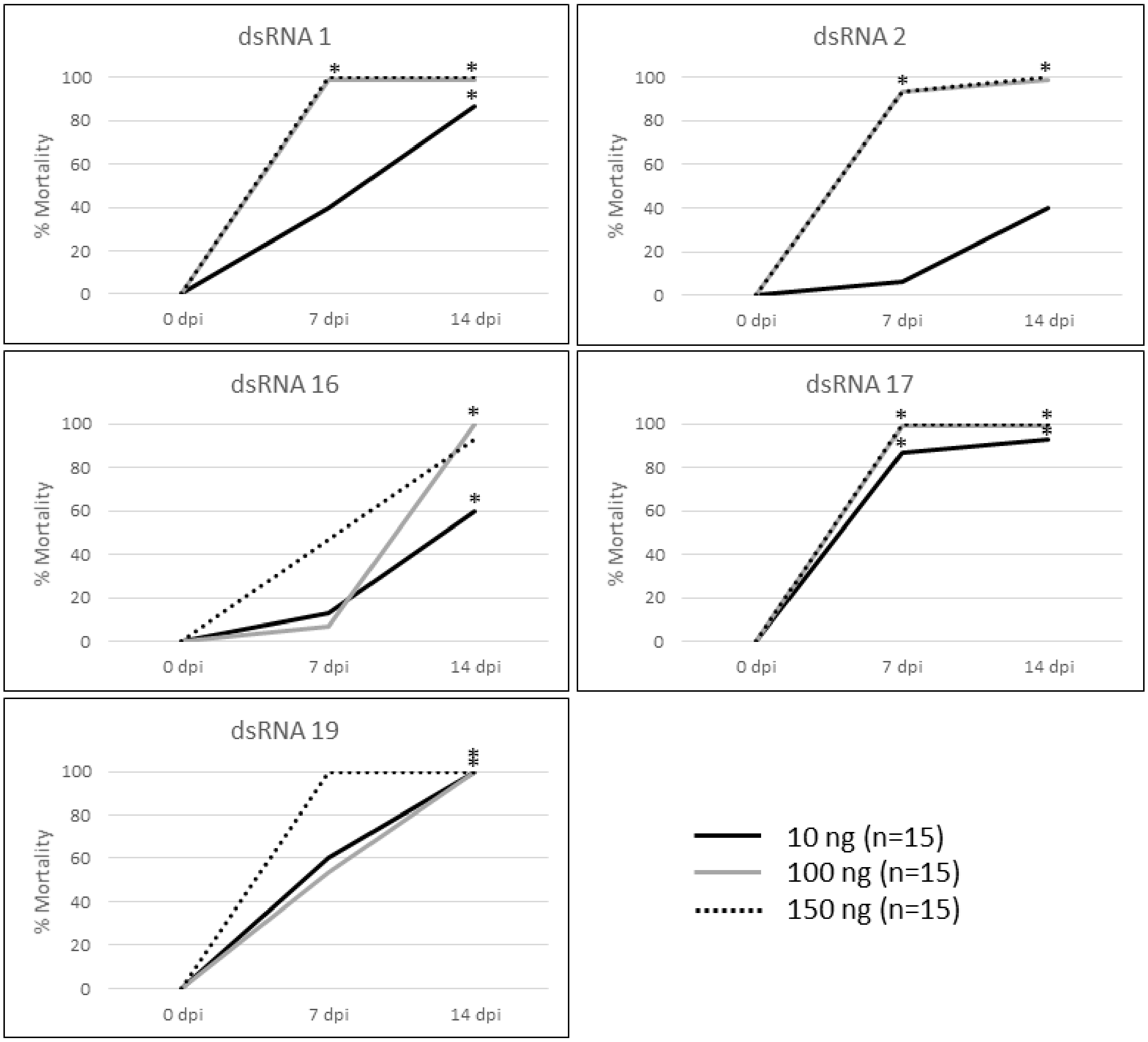
dsRNA	Targeted Gene Product	# of Survivors			
		2nd Instar (out of 5)	Adult (out of 3)		
		7 dpi	7 dpi	14 dpi	21 dpi
1[a]	β-actin	0	3	2	2
2[a]	actin	0	3	3	2
3	chitin synthase	5	3	3	3
4	trypsin-like serine protease	5	3	3	3
5	trypsin-like serine protease	5	3	3	3
6	chitin deacetylase	5	3	3	3
7	chitin deacetylase	4	3	3	3
8	G12-like protein	4	3	3	3
9	peritrophic membrane protein	4	3	3	3
10	peritrophic membrane protein	3	3	3	3
11	zinc carboxypeptidase	3	3	3	3
12	peritrophic membrane protein	5	3	3	3
13	serine protease	4	3	3	3
14	unknown	5	3	3	3
15	peptidase	5	3	3	3
16[a]	V-type proton ATPase	1	3	2	0
17[a]	actin	0	2	2	1
18	peritrophic membrane protein	3	3	3	3
19[a]	polyadenylate binding protein	0	3	0	0
21	helicase	5	3	3	3
22	myosin heavy chain protein	3	3	3	3
23	α-amylase	5	3	3	3
24	unknown	5	3	3	3
CTL	Plant virus gene	5	3	3	3
--	Injection dye only	5	3	3	3

[a] selected for further investigation

The five dsRNAs that were selected for further investigation based on the preliminary trials were evaluated using different CRB life stages. All five induced high mortality in these life stages at 14 days post injection (dpi) when 150ng of dsRNA was used. Control treatments (plant virus dsRNA and loading dye only) induced mortality rates <13% at 14 dpi (data not shown):



Injection of 2nd instar with 100 or 10ng of dsRNA also led to elevated mortality, indicating a relatively low dosage of certain dsRNAs (dsRNA 17 in particular) is capable of inducing mortality (astericks indicate significantly higher mortality over control treatments):



OBJECTIVE 3. ENGINEER AND DEPLOY SMART TRAPS.

Originally our planned approach was to build a surveillance system around the Intel Edison, a postage-stamp sized dual-core microcomputer system, with off-the-shelf USB cameras through a customized interface. This choice seemed logical given the versatility of the powerful Linux based operating system to implement open source machine vision tools such as from OpenCV (also developed by Intel), and small footprint. Even so early on in this project year we made the decision to switch to a different microcomputer system due to the discontinuation of the Intel Edison, and we believe the switch will allow versatile surveillance systems to be developed with a smaller footprint, greater energy efficiency, and significantly more affordable cost.

We are currently developing machine vision systems on microcomputer systems built around the ESP32 microcontroller from Espressif Microsystems. The ESP32 is an upgraded version of Espressif's highly successful ESP8266 microcontroller, which we have already used successfully to develop a handheld open-source potentiostat for versatile electrochemical analyses, the first of its kind in that is capable of full spectrum electrochemical impedance spectroscopy up to 100 kHz and user-defined bias. The low cost, many versatile features, and large user community of these single chip microcontrollers make it unlikely that they will be discontinued as was the platform we were using in our initial development.

In addition to the built-in WiFi capability and limited general-purpose input/output (GPIO) of the ESP8266, the ESP32 includes embedded Bluetooth capability (including classic Bluetooth and the more recent "low energy" Bluetooth standard), and an expanded set of GPIO that can be directly interfaced to small and highly affordable digital camera modules as well as audio codecs for acoustic based surveillance. Interestingly, Espressif has done pioneering work developing a "long range" (up to 1 km) WiFi communications standard that can be enabled on the ESP32, which could be used in a WiFi mesh network to greatly extend range of remotely deployed surveillance systems where a local internet access point is not available, without using satellite or other radio communication subscription services or any additional hardware dependencies.

We are currently working with a highly compact off-the-shelf ESP32 module with embedded camera (M5Stack ESP32 Camera, available for about $10) to implement software routines to capture images and upload through WiFi to a server offsite. While the Espressif software includes rudimentary face recognition libraries, for more robust recognition of "objects" such as CRB with more limited training data our current approach is to use the ESP32 merely to send images of interest to a central server to be analyzed with more powerful GPU. Since we first began working on this project, Google's platform "TensorFlow" has rapidly become the machine vision tool of choice, with a variety of high performance trainable convolutional neural networks. We are currently leveraging TensorFlow training and implementation workflows developed for rapidly identifying the invasive tree *Miconia calvescens* in aerial images, to develop analogous models for identifying CRB in traps in the field.

As our approach relies on periodically uploading images from remotely deployed traps, we are also beginning to explore the implementation of the long range WiFi (802.11) mode into a mesh network to relay images back to an internet access point. While this protocol inherently operates at a much lower data rate than traditional WiFi, we don't anticipate this to be a problem as images will be relatively low resolution (highest available resolution 1600 x 1200 pixels, though we expect that even lower resolutions will be sufficient for our application), and only uploaded intermittently (i.e. once per day, and in response to other events of interest such as acoustic recognition of wingbeat characteristic of CRB).

The simplest approach for surveillance, especially for panel traps where captured CRB remain in view of the camera, is to have the system wake up once per day from a low power "sleep" mode, record and upload an image. Even so we are working on more customized hardware designs to incorporate a variety of other peripherals that could be useful for improved surveillance. This includes use of chip level microphones that can interface directly with ESP32 through i2s protocol, and GPIO pins that can be configured to be used as capacitive "touch sensors". The touch sensor approach could be a very easy way to detect events of interest like CRB falling into a trap with a single wire positioned at any point(s) of interest, as opposed to approaches like interruption of a light beam where positioning source and detector is more difficult and significantly greater amount of hardware overhead and assembly time is required. In addition, some degree of customization is required to incorporate connector for rechargeable battery and photovoltaic panel input, and charging circuit to enable uninterrupted operation in the field. Our objective is to develop a fully-functional small footprint (<2" square) fully integrated system, that can be deployed for less than $100 per trap including solar panel, battery, and commercial fabrication and assembly costs at the scale required for this project.

Lighting preference experiments for CRB

Leveraging funding from other projects, including support from HISC, we previously developed a prototype programmable lighting system to evaluate lighting preference for ACP to improve efficacy of trapping in Smart-Traps. The lights could be easily programmed with an Android phone to turn any of 6 LEDs (UV, Blue, Green, Yellow, Amber, or Red) for programmable durations (before sunrise, after sunset, or all night) with various available modulation schemes (i.e. flashing at a user defined frequency). In preliminary forced choice tests with lab-reared CRB (that do not have opportunity to fly or forage) beetles consistently moved into unlit portions of a tunnel and away from lighted portions no matter the color of light. While these observations suggest that CRB can perceive light from near-UV all the way through the visible spectrum, we believe that results are inconclusive about whether wild foraging CRB are attracted to artificially lit traps. We also attempted limited field testing in panel traps on Oahu with this lighting design, though results of these tests were also inconclusive due to limited amount of data (i.e. deployment of lights in only a handful of traps, and low numbers of trap catches on Oahu). In addition, the field performance of the preliminary lighting design was sub-optimal (i.e. energy management was relatively inefficient and there was no mechanism to prevent overdischarge / killing of the rechargeable batteries).

To facilitate more systematic field testing in the last year we redesigned the programmable lighting system to operate with much more efficient energy regulation, programmed devices to enter an extremely low power "sleep" mode when lights are off, and included a low battery shutoff to prevent overdischarging of batteries. These modifications have greatly extended the operating life of the device especially for situations where PV recharging is impractical or limited by excessive shade- so for example lights could be operated for 10 days operating high power LEDs two hours per night on a single charge, enabling much easier evaluation of lighting preference in the field. While currently 6 panel traps have been tested with these lights on Oahu, we have also sent 60 of the lights with PV panels and batteries to a collaborator on Guam for testing there where population pressures are much higher. Unfortunately deployment of these lighting preference experiments on Guam have been delayed by a series of severe weather events and associated recoveries through Fall of 2018.

Instrumented "dumpster" trap

Our collaborators at USDA-WRRC (Western Regional Research Center, Albany CA) have developed a prototype "dumpster" style trap to test on Oahu, operating on the principle that the trap can be filled with nesting and feeding materials that are highly attractive to CRB, and that the resulting concentration of aggregation pheromones and other chemical communication can synergize to improve trapping effectiveness. The trap is also arrayed with relatively large photovoltaic panels and integrated electrical systems to facilitate deployment of lighting and surveillance systems to begin gathering more field data.

We received the trap in March 2019, and deployed it in the field near a known CRB breeding site in April. On a recent check the trap contained six beetles illustrating preliminary success with the design approach, though we failed to acquire any images of CRB using a commercially available wildlife camera (with IR-sensor motion-triggered shutter). We will be redeploying the trap again shortly, and attempt to install prototype version of our miniature WiFi surveillance system shortly to teset in the field.