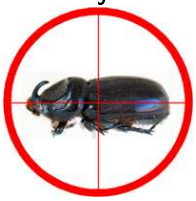
Final Report for US Forest Service Grant 11-DG-11052012-101

Performance Period: 23 May 2011 - 31-December 2013



Support for the Guam Coconut Rhinoceros Beetle Eradication Project

Prepared by Aubrey Moore, University of Guam

Wednesday 23rd April, 2014

Contents

At a Glance: Progress Since the Last Semiannual Report (Nov. 2013)

- Chemical Control
  - We continued a field trial in which we sprayed crowns of coconut palms with cypermethrin indicates that this treatment kills adults and protects plants from further damage.
- Biological Control
  - We are continuing to import green muscardine fungus spores from the Philippine Coconut Authority and are applying this biocontrol agent to breeding sites.
  - Dr. Sean Marshall, a collaborator from AgResearch New Zealand, visited Guam in January to set up bioassays to test new strains of ORNV for biocontrol of CRB on Guam and to determine if the Guam CRV population is resistant to ORNV strains which are pathogenic to other CRB populations in the Pacific.
- Improved Trapping
  - A recent field trial indicates that addition of solar powered ultraviolet light emitting diodes to standard CRB pheromone traps increases trap catch by more than 2X.
  - We have developed a novel barrel trap which is an artificial breeding site contained in a 55 gal. drum. A chicken wire top allows CRB adults to enter, but prevents them from flying out. A recent field trial indicates that barrel traps catch more than 10X more beetles than surrounding standard CRB pheromone traps. Recent experiments have shown a 25% escape rate throught the chicken wire. New barrel traps have been designed to prevent escape.
- Other Eradication Project Support
  - We recently developed a new extension flyer5 on CRB management and held public workshops for pest control professionals and the public.
  - Following interception of a CRB adult in the Honolulu International Airport on November 20, 2013 followed by a CRB adult caught in a pheromone trap at Hickam Air Force Base on December 23, 2013, Aubrey Moore and Roland Quitugua were asked by APHIS and Hawaii DOA to assist in planning an eradication attempt. Moore and Quitugua spent a week in Honolulu acting as subject matter experts. There was no expense to the Forest Service project for this assistance. However, Hawaii is benefitting from research supported by by Forest Service grants. The Hawaii project has adopted improved trapping methods developed on Guam including addition of ultraviolet light emiiting diodes (UVLEDs) to pheromone traps and use of barrel traps (artificial breeding sites).
  - An online bibliography of scientific literature on the coconut rhinoceros beetle was established at http://guaminsects.myspecies.info/crb-biblio.

Eradication is the ultimate long-term objective of the Guam Coconut Rhinoceros Beetle (CRB) Eradication Project. Implementation of chemical and biological control to suppress the population and prevent an imminent outbreak of CRB adults is our short-term objective. If eradication is cannot be realized, this work will lead towards integrated pest management for CRB on Guam.

1 Chemical control

1.1 Evaluation of cypermethrin and insect growth regulators applied as drench treatments for control of CRB in compost piles and other breeding sites

Cypermethrin, the only active ingredient found to be efficacious in laboratory bioassays, is currently being field tested as a drench. Several insect growth regulators are currently being tested in lab bioassays. Our objective is to publish well-documented extension recommendations that landscape managers at hotels, parks, and golf courses can use to prevent generation of adult beetles in large compost piles.

Percent Complete: 90%

Progress:

- Laboratory bioassays indicated that the insect growth regulator, pyriproxyfen, prevents pupation of *Oryctes rhinoceros* grubs
- The project's Environmental Assessment (EA) was updated to include cypermethrin and pyriproxyfen as drench treatments for compost piles and other sites infested with *O. rhinoceros* grubs. The EA was published in December, 2011 and resulted in a Finding of No Significant Impact in February 2012.
- A large scale field trial was established at Oka Point to test drench treatments of cypermethrin and pyriproxyfen. Note that the generation time for rhino beetles on Guam is about nine months. Therefor, field trials can be expected to last for several months.

- Pest control operators on Guam are currently spraying crowns of coconut palms with cypermethrin and claim to be killing lots of adults as evidenced by dead beetles found beneath trees the following day.

- After learning that some pest control operators on Guam are attempting to protect high value ornamental palms from CRB damage by spraying crowns with cypermethrin, we decided to test this method as a valid IPM tactic. We applied biweekly spray applications of cypermethrin to the crowns of 32 young coconut palms along the entrance road to the University of Guam Agricultural Experiment Station at Yigo, Guam. As a damage index, we counted how many of the youngest four fronds on each tree showed signs of CRB damage. The damage index fell from 4.00 to 0.62 during 5.5 months of treatment. Spray residue collects at the base of petioles which is the site at which CRB initiates bore holes. In daily inspections of the ground under each treated palm, we found 29 dead or dying CRB adults, indicating that they are knocked down prior to boring into the crowns. (See Appendix ?? for details).

To Do:

- Analyze results from Oka Point field trial..
- Write and publish extension information on chemical control of rhino beetle grubs.
- Publish results in a scientific journal.

1.2 Evaluation of SPLAT RB plus 5% cypermethrin as an attracticide for CRB adults

SPLAT RB is a product manufactured and marketed by ISCA Technologies Inc. SPLAT RB is the CRB pheromone that we currently use, infused into a sticky matrix. I am working in collaboration with ISCA to evaluate an attracticide made by adding 5% cypermethrin. The concept is simple: Adults, both males and females, are attracted to the SPLAT, make physical contact, and pick up a lethal dose of cypermethrin. Preliminary lab bioassays and semi-field trials in a large (20 ft x 40 ft) field cage indicate that this idea might work.

By applying blobs of the RB SPLAT to the crowns of coconut palms, it may be possible to protect high value trees, killing

adults before they make bore holes. Thus, preventing damage. Results from large field cage experiments will be published in a peer reviewed journal and extension recommendations will be published if results are encouraging.

Percent Complete: 70%

Progress:

- Original field cage was abandoned because of an unacceptably high escape rate for test insects. As a replacement, two large field cages (20' x 20' x 10') were designed, custom manufactured, and installed at the University of Guam Yigo Agricultural Experiment Station.
- Semi-field evaluation of SPLAT has begun in these cages. Preliminary results indicate that beetles are attracted to the SPLAT target, but very few make physical contact necessary for intoxication. It is possible that the pheromone release rate is too high.
- Note that experiments involving beetle flight can only be performed with during the flight period for rhino beetles which is just after sunset, on nights with light wind and no rain, and on nights when project personnel are available.
- In preliminary large field cage experiments, very few adults were killed by RB SPLAT plus cypermethrin. Tracer dye washed from beetles indicated that very few beetles made physical contact with the formulation. It is possible that the pheromone release rate is too high, causing beetles to become arrested or repelled prior to physical contact with the SPLAT. Note that there is strong evidence that the release rate from the ChemTica pheromone lures used in our standard traps is too high, and the release rate of the pheromone from the SPLAT appears to be even much higher than this.
- See **??** for experimental details.

To do:

- Use video cameras to document behavior of beetles flying near SPLAT targets.
- Test at lower pheromone release rates.

2 Biological control

2.1 Establishment of *Metarhizium majus* as a biological control agent for CRB

Metarhizium majus, formerly known as *Metarhizium anisopliae* (var. *majus*) is a soil inhabiting fungus which is virulent against CRB and other scarabs. It persists in CRB habitat and can be autodisseminated by the beetle. *M. majus* has been used as a successful biocontrol agent for CRB by the Philippine Coconut Authority (PCA) for several years. PCA grows the fungus on sterile, cooked corn and sells this to farmers to add to CRB breeding sites within their coconut plantations.

Pending receipt of a USDA-APHIS permit to import *Metarhizium*, I will visit with Dr. Ambrose Alfiler at the PCA to learn how to culture the fungus and how to use it for CRB biocontrol.

Percent Complete: 100%

Progress:

- An APHIS permit to import *Metarhizium* from the Philippine Coconut Authority was approved
- The projects EA was updated to include use of *Metarhizium*.
- Aubrey Moore visited Ambrose Alfiler's lab in the Philippines in September 2011. *Metarhizium* spores brought back to Guam were found to be highly pathogenic for Guam rhino beetles in lab bioassays. We also tested closely related *Protaetia* scarab grubs and found that these were unaffected by the spores.
- To date, six 15-kg shipments of *Metarhizium* spores have been imported. These have been deployed in 3 ways:
  - incorporation into natural rhino beetle breeding sites
  - incorporation into artificial rhino beetle breeding sites ("sinks")
  - autodissemination by dust male beetles caught in traps with spores and subsequently releasing them

- Prior to introduction of *Metarhizium*, we found no evidence of biological control by this entomopathogen in thousands of grubs examined. We now find infected grubs in areas distant from those directly treated with spores, indicating that autodissemination is occurring.

2.2 Determination of reasons why virus failed to control CRB on Guam

It is of regional importance to determine why we have been unable to kill Guam rhino beetles using eight strains of virus produced by Dr. Trevor Jackson's lab in New Zealand. Virus has been very effective in limiting population density and damage caused by CRB on Pacific Islands over the past 50 years. Perhaps the Guam beetles come from a resistant population. Resistance to the virus would explain the resurgence of rhino beetles in Palau, where virus biocontrol has been used for many years. An alternate cause of failure could be a loss of virulence in the New Zealand lab strains, which are grown in insect cell culture.

I have a USDA-APHIS permit to import live, adult rhino beetles from susceptible populations. I plan to perform laboratory bioassays which will compare susceptibility of the Guam beetles with those from susceptible populations. This work will be performed in collaboration with Dr. Sean Marshall and Dr. Trevor Jackson, AgResearch, New Zealand.

Percent Completion: 50%

Progress:

- This objective will receive continuing support by a new USDA-APHIS biocontrol grant in collaboration with Trevor Jackson, AgResearch, New Zealand. The project has already been approved and detailed plans were finalized at meeting with Aubrey Moore, Russ Campbell, Trevor Jackson, and Sean Marshall at the

Pacific Plant Protection Organization meeting in Fiji, June 2012. New virus samples were provided by AgResearch and lab bioassays were performed on Guam.

- No pathogenic effects were observed in bioassays using the new virus samples, further supporting the hypothesis that the Guam population is resistant to the virus. See **??** for experimental details.
- Bioassays with new virus strains are currently being conducted.

To Do:

- Determine why the virus does not kill Guam's CRB population.
- Find a strain of virus which is efficacious for Guam's CRB population.

3 Improved Trapping

We know that the standard baffled bucket traps baited with oryctalure pheromone which are used by the project are inefficient from two lines of evidence. Firstly, coconut palms are repeatedly damaged in mass trapping areas, indicating that the palms are more attractive than the traps. Secondly, in a preliminary mark-release-recapture experiment in which 20 adult CRB were released in a mass trapping area, not a single beetle was recaptured. We will perform the following studies to see if we can find out how to improve trap performance.

3.1 Determine if adult CRB escape from traps

The literature states that adult CRB are unable to escape from the standard trap design we are using because they require a lot of open space for take-off. However, on several occasions, we have observed CRB taking off vertically ('helicoptering'). We will place CRB selected for flight propensity in traps inside our large field cage to see if any escape.

Percent Complete: 100%

Progress:

In repeated large scale field cage tests. no beetles escaped from standard design baffled bucket traps. See **??** for experimental details.

3.2 Observation of CRB flight activity in vicinity of traps

We will perform large field cage and field experiments to observe flight behavior in the vicinity of pheromone traps. We plan to employ visual observation, infrared trail cameras, and radio tracking in these experiments. We already have eyeballs and an IR trail camera. Radio tracking equipment is on loan from the USGS brown treesnake project. However, we need to purchase miniature radio tags designed for tracking insects.

Percent Complete: 25%

Progress:

- Preliminary large field cage experiments with standard vaned bucket traps indicate that traps bated with fresh lures and depleted lures are equally attractive.
- A motion-sensitive infrared trail camera has been tested and it will trigger and make images of rhino beetles flying in the dark
- Radiotelemetry transmitters have been ordered
- Note that experiments involving beetle flight can only be performed with during the flight period for rhino beetles which is just after sunset, on nights with light wind and no rain, and on nights when project personnel are available.
- Large field cages are currently being repaired following minor damage from high winds.

3.3 Semiochemical experiments

In collaboration with two chemical ecologists, Dr. Eric Jang, USDA-ARS Pacific Basin Research Center, and Dr. Gadi Reddy, Western Pacific Tropical Research Center, University of Guam,

we will perform semiochemical experiments to see if we can improve trap catch. Planned experiments include characterizing and evaluating a new CRB attractant we have discovered, and optimizing pheromone release rates.

Percent Complete: 98%

Progress:

- A team of insect chemical ecologists under the leadership of Eric Jang and Matt Siderhurst, USDA-ARS Pacific Basin Research Center visited Guam during May 2012 and again during October and November, 2013. The team used an olfactometer and an electroantennagram to test potential natural and artificial semiochemicals which could be used to modify rhino beetle behavior. Candidate compounds where also characterized using GC-MS instrumentation.

- The project is shipping live rhino beetles to Eric Jang at PBARC under an APHIS import permit. These beetles are being used to continue electroantennagram studies. In addition to working on semiochemicals, our Hawaiian collaborators have been investigating the use of light emitting diodes to improve trap catch.

- In large field cage experiments traps with depleted lures (all liquid pheromone evaporated) trapped equal amounts of beetles as did traps equipped with fresh lures, indicating that the release rate of the lures is too high. This hypothesis is further supported by the observation that traps deployed in the island-wide trapping caught more than twice as many beetles during trapping periods immediately prior to lure replacement. See **??** for experimental details.

- A field trial was conducted to test increased attractiveness of standard CRB pheromone traps by addition of ultraviolet light emitting diodes (UVLEDs) and use of reduced release rate lures. UVLEDs increased the trap catch rate by almost 3X when used in conjunction with pheromone lures. Only 2 CRB were caught in traps equipped with a UVLED but without a pheromone lure, indicating that the light sources act synergistically with pheromone lures. Our use of inexpensive solar powered UVLEDs is novel. There was no significant difference in trap catch rate between traps equipped with standard and reduced release rate lures, even though the release rate was reduced by an average of 90%. See **??** and **??** for experimental details,

- Barrel traps are artificial CRB breeding sites contained in used 55 gallon oil barrels or similar sized containers. A chickenwire cover allows adult beetles to land on the trap and fall into it. But they cannot escape because the chicken wire prevents them from flying out. The capture rate for barrel traps is more than a magnitude higher than that of surrounding standard CRB pheromone traps. Trap capture

rate can be further increased by more than 50% by addition of solar powered ultraviolet light emitting diodes. See ?? for experimental details.

To Do:

- Test the new sample of "Body Butter" as a rhino beetle attractant.

4 Other Eradication Project Support

Funds will be used to support and improve ongoing eradication activities including:

- Pheromone trapping
- Maintenance of the project's georeferenced, online database
- Surveillance by human and canine scouts
- Sanitation to remove CRB breeding sites
- Maintenance of detector dogs and associated facilities
- Maintenance of a CRB rearing facility to produce beetles for autodissemination and research

Percent Complete: 100%

Progress:

- Trapping records and other project data are stored in an online, georeferenced database. Summary statistics for any time period can be accessed at http://guaminsects.net/oryctes/stats.php.
- During the performance period for this grant, May 23, 2011 until present, 1040 pheromone traps distributed throughout the island were maintained and operated. The US Navy provided personnel for trapping on the Naval Base. All trap data were stored on the project's georeferenced, online database. Since start of performance period for this project 27,388 trap visits were made and 8.160 adult beetles were trapped. The infestation has spread to most parts of the island. However, average trap catch is relatively low (less than 0.02 beetles per trap-day) (Figure 1).

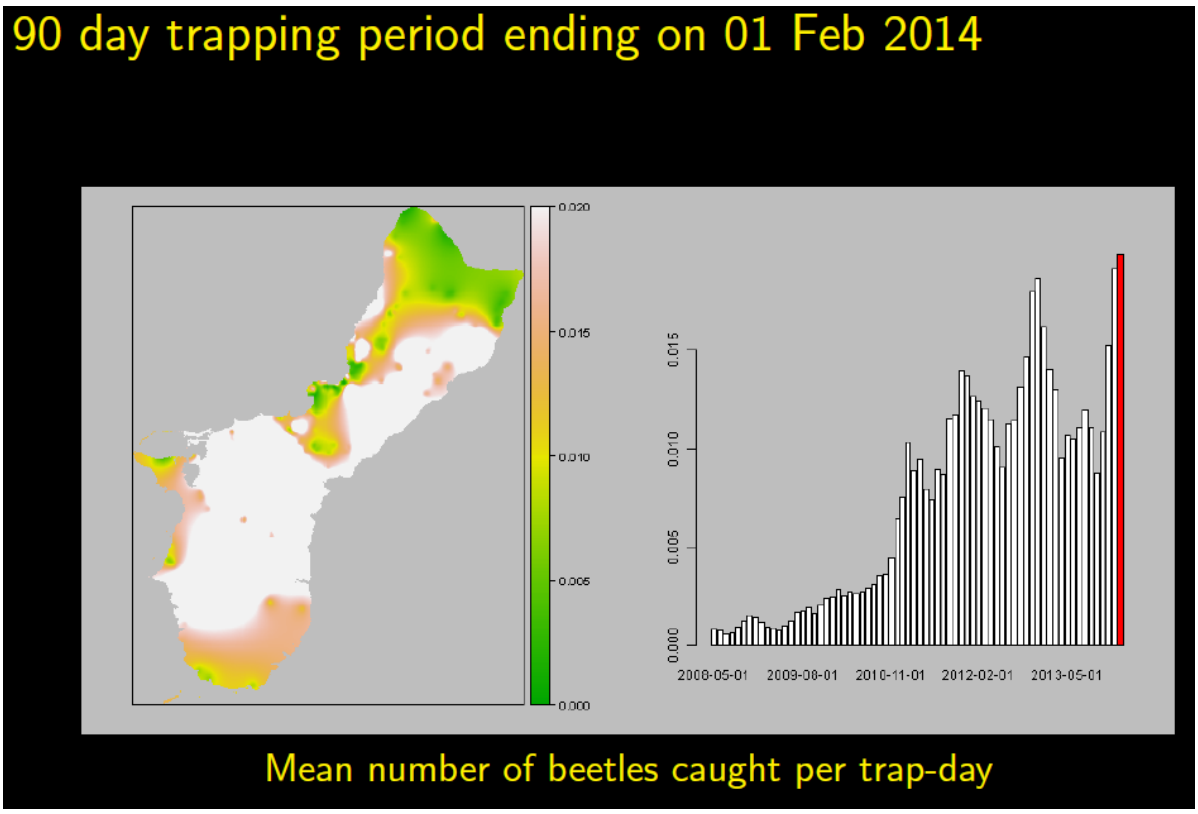


Figure 1: Spatial-temporal display of coconut rhinoceros trap data. This is the last frame from a time series. The entire series can be viewed at http://guaminsects.net/anr/content/visualization-coconut-rhinoceros-beetle-trap-catch-data.

- The project's sanitation crew found and destroy 1,641 adult beetles and 13,278 immatures. Eighty-eight dead or dying coconut palms were felled and destroyed to prevent them from turning into breeding sites.
- The project's canine section (4 dogs and 4 dog handlers) was disbanded in November 2011 because of uncertainties in future funding and reduced relevance following spread of the infestation from geographically isolated spots to coverage of most of the island. During August 2011 through November 2011 the dogs discovered 106 rhino beetle breeding sites.
- The project insect rearing facility is operating well and is keeping up with demands for experimental animals. Live beetles are shipped to collaborators in Hawaii once per month following protocol specified by an APHIS permit. Freshly trapped male adult beetles are currently being used for autodissemination of *Metarhizium* instead of reared individuals.

5 Appendix: Trifold Flyer: CRB Control Tips

Latest version can be downloaded from http://guaminsects.net/anr/sites/default/files/reducedrhino%20brochure3.pdf

Simple CRB Trap made with recycled materials

A basic trap can be made using a metal barrel with a chicken wire top.

Compost material is placed in the bottom of the barrel to attract beetles to breed and lay eggs. The chicken wire allows beetles to enter, but they cannot exit as their open wings prevent them from passing through the wire.

It is important that the compost material is kept at least 6 inches below the top of the barrel to prevent beetles from crawling out.

CRB BIOCONTROL

Green Muscardine fungus (GMF) is an effective biocontrol agent that targets the adult and larval stages of CRB. This strategy has been found effective for controlling the rhino beetle population on Guam.

Larva infected with the green Muscardine fungus

CONTROL TIPS

- clear all green waste including dead palm trees, stumps and trunks
- manage coconut trees by removing dead fronds & inflorescenses
- monitor compost piles for larvae and destroy any larvae found
- apply green Muscardine fungus to organic waste piles, compost piles and gardening beds

To Report Sightings Call:

475-PEST (7378)

Prepared by:

Dr. Aubrey Moore
Roland Quituqua
Olympia Terral
(671) 735-2086

University of Guam
Cooperative Extension Service, ANR

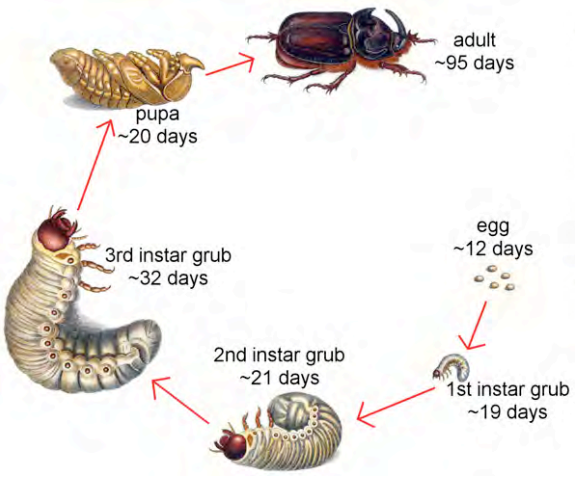
rev. July 10, 2013

Coconut Rhinoceros Beetle

Control Tips

This brochure was made possible through grants from the USDA Forest Service, USDA-APHIS, and the Guam Legislature.

CRB LIFE CYCLE



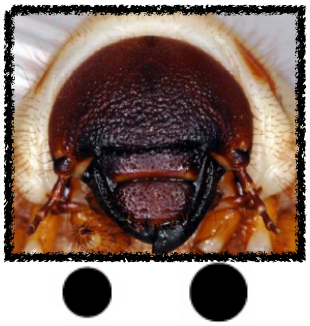
The coconut rhinoceros beetle (CRB), *Oryctes rhinoceros*, is a large scarab beetle that feeds on coconut and other palms. The adult beetles bore holes into the crowns of coconut trees and feed on the sap. This is what causes the distinctive v-shaped cuts in the leaves.

Rhino beetles have 4 life stages: eggs, larvae, pupae and adults. The female rhino beetle lays her eggs in decaying logs and other organic matter. Only adults cause damage. However, it is very important to remove dead coconut trees and other organic material from your yard and surrounding areas before adults develop.

CRB LARVAE

CRB's rough head capsule distinguishes it from other scarb beetle grubs on Guam.

1st instar
black dots represent the size range of head capsule 2.5 - 3.1 mm

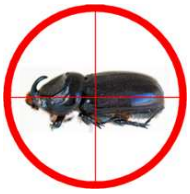


3rd instar
black dots represent the size range of head capsule 9.5 - 11.2 mm

2nd instar
black dots represent the size range of head capsule 5.0 - 6.0 mm

6 Appendix: 2013-11-05 Cypermethrin Applied to Coconut Palm Crowns as a Propholactic Treatement for Prevention of CRB Damage

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Cypermethrin Applied to Coconut Palm Crowns as a Prophylactic Treatment for Prevention of CRB Damage

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

November 5, 2013*

After learning that some pest control operators on Guam are attempting to protect high value ornamental palms from CRB damage by spraying crowns with cypermethrin, we decided to test this method as a valid IPM tactic. We applied biweekly spray applications of cypermethrin to the crowns of 32 young coconut palms along the entrance road to the University of Guam Agricultural Experiment Station at Yigo, Guam. As a damage index, we counted how many of the youngest four fronds on each tree showed signs of CRB damage. The damage index fell from 4.00 to 0.62 during 5.5 months of treatment. Speay residue collects at the base of petioles which is the site at which CRB initiates bore holes. In daily inspections of the ground under each treated palm, we found 29 dead or dying CRB adults, indicating that they were knocked down prior to boring into the crowns.

*Revised February 16, 2014
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/crownSpray/crownSpray

Figure 1: Applying cypermethrin to crowns of young coconut trees.

1 Methods

A row of 32 young coconut palms planted along the entrance road to the University of Guam Agricultural Experiment Station in Yigo were sprayed with cypermetherin on a biweekly schedule (Table 2, Figure 1). These trees ranges from 8 to 20 feet in height. As an index of CRB damage, I count how many of the four youngest fronds had distinctive CRB damage. If a spear (an unopened frond) was present, this was considered to be the youngest frond. Damage assessments were performed at the start of the experiment on May 19, 2013 and on November 5, 2013. I checked for and collected daead or moribund CRB adults under each tree each morning.

2 Results and Discussion

All trees were very heavily damaged at the start of the experiment. All of the youngest four fronds on each tree bore signs of CRB damage (Table 1). Thus, the average damage index, on a scale of 0 to 4, was 4.000.

When the trees were observed 5.5 months later, the average damage index had dropped to 0.625. Eighteen of the 32 trees (56%) had none of their four newest fronds damaged and only one tree had all four new fronds damaged.

During the same 5.5 month period, 29 dead or dying beetles were collected beneath the treated trees.

This study was more of an emergency control operation than an experiment. Because we did not reserve untreated trees as an experimental control, we do not know if the reduced damage to new fromds is in response to the cypermethrin applications. However, this is probably the case, because we did observe mortality of adult beetles attacking the treated trees. Because cypermethrin has a quick knockdown effect, as with most pyrethroids. It is likely that the beetles were intoxicated shrtly after arriving and before they were able to bore into the crown. It should be noted that when the canopy is sprayed, the liquid runs down the inside of the petioles and collects at the angle between the petioles and the trunk at the location were CRB initiate their bore holes.

Table 1: CRB damage index (number of four youngest fronds damaged).

	tree	damage20130519	damage20131105
1	3434	4	0
2	3433	4	1
3	3432	4	0
4	3431	4	1
5	3430	4	2
6	3429	4	2
7	3428	4	1
8	3427	4	1
9	3425	4	0
10	3424	4	0
11	3423	4	1
12	3422	4	1
13	3421	4	0
14	3420	4	1
15	3419	4	0
16	3418	4	1
17	3417	4	0
18	3416	4	0
19	3415	4	0
20	3413	4	1
21	3412	4	0
22	3411	4	0
23	3410	4	4
24	3409	4	0
25	3408	4	1
26	3407	4	0
27	3406	4	0
28	3405	4	0
29	3404	4	0
30	3403	4	2
31	3402	4	0
32	3401	4	0

Table 2: Cypermethrin treatments.

	date	application
1	2013-05-18	Demon Max; Â½ oz per gal; 50 gal; no spreader/sticker
2	2013-06-14	Demon Max; Â½ oz per gal; 40 gal; no spreader/sticker; rained later in day
3	2013-07-01	Demon Max; Â½ oz per gal; 40 gal; no spreader/sticker
4	2013-07-15	Demon Max; 1 oz per gal; 40 gal; spreader/sticker
5	2013-07-29	Demon Max; 1 oz per gal; 40 gal; spreader/sticker
6	2013-08-12	Demon Max; 1 oz per gal; 40 gal; spreader/sticker
7	2013-08-26	Demon Max; 1 oz per gal; 40 gal; spreader/sticker
8	2013-09-09	Demon Max; 1 oz per gal; 40 gal; spreader/sticker
9	2013-09-23	Demon Max; 1 oz per gal; 40 gal; spreader/sticker
10	2013-10-07	Demon Max; 1 oz per gal; 40 gal; spreader/sticker
11	2013-10-21	Demon Max; 1 oz per gal; 40 gal; spreader/sticker
12	2013-11-04	Demon Max; 1 oz per gal; 40 gal; spreader/sticker

Table 3: Beetles found beneath sprayed trees.

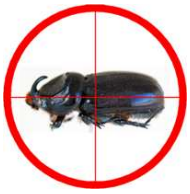
	date	tree
1	2013-05-19	3418
2	2013-05-19	3427
3	2013-05-19	3428
4	2013-05-19	3431
5	2013-05-19	3417
6	2013-05-21	3433
7	2013-05-21	3418
8	2013-05-22	3412
9	2013-05-23	3407
10	2013-05-26	3407
11	2013-05-28	3427
12	2013-06-04	3407
13	2013-06-04	3413
14	2013-06-08	3430
15	2013-06-14	3407
16	2013-06-17	3406
17	2013-06-17	3432
18	2013-06-22	3401
19	2013-07-06	3403
20	2013-07-23	3411
21	2013-08-02	3434
22	2013-08-10	3401
23	2013-08-10	3431
24	2013-08-13	3417
25	2013-09-03	3416
26	2013-09-15	3410
27	2013-09-20	3429
28	2013-10-12	3406
29	2013-10-12	3410

Table 4: Number of dead or moribund beetles found under each tree.

	tree	nbeetles
1	3401	2
2	3403	1
3	3406	2
4	3407	4
5	3410	2
6	3411	1
7	3412	1
8	3413	1
9	3416	1
10	3417	2
11	3418	2
12	3427	2
13	3428	1
14	3429	1
15	3430	1
16	3431	2
17	3432	1
18	3433	1
19	3434	1

7 Appendix: 2013-11-06 Development of Barrel Traps

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Development of Barrel Traps

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

November 6, 2013*

Barrel traps are artificial CRB breeding sites contained in used 55 gallon oil barrels or similar sized containers. A chickenwire cover allows adult beetles to land on the trap and fall into it. But they cannot escape because the chicken wire prevents them from flying out. The capture rate for barrel traps is more than a magnitude higher than that of surrounding standard CRB pheromone traps. Trap capture rate can be further increased by more than 50% by addition of solar powered ultraviolet light emitting diodes.

1 Methods

Barrel traps are artificial CRB breeding sites contained in used 55 gallon oil barrels or similar sized containers (Figure 1).The barrel is loaded with decaying coconut material from a natural CRB breeding site containing all CRB lifestages. A chickenwire cover allows adult beetles to land on the trap and fall into it. However, beetles cannot escape because the chicken wire prevents them from flying out.

We deployed 24 barrel traps in the back yards of cooperators and visited these weekly. We placed an oryctalure pheromone dispenser in each trap when first installed. Initially, we censused all beetles in the trap by going through the breeding material. However this was very time consuming. The traps were modified by placing a galvanized or plastic pan underneath the chicken wire to capture

*Revised February 16, 2014
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/barrelTraps

newly arrived adults (Figure 2). Small holes drilled in the pan allow passage of odors emitted by the breeding material. During our weekly trap visit, we count and sex beetles in the pan and then dump them into the breeding material. When the breeding material has become depleted, we add several "pucks" which are 2 inch thick slices of rotting coconut logs.

We compared the trap catch rate of each barrel with those of standard CRB pheromone traps within a one km radius. We tested the utility of placing solar powered ultraviolet light emitting diodes (UVLEDs) on our barrel traps by placing them on a randomly selected half of our traps for a week, switching them to the other half of the traps on alternate weeks.

2 Results and Discussion

- Barrel traps caught a mean of 0.211 beetles per trap-day. In comparison, the mean capture rate for standard CRB pheromone traps within a one km radius of the barrel traps was 0.016. The difference is highly significant (p-value = 5.919e-7; Welch Two Sample t-test). Thus, the barrel traps caught 13X as many beetles as the standard traps.

- Barrel traps fitted with solar powered UVLEDs captured 0.246 beetles per trap-day. In comparison, barrel traps without UVLEDs captured 0.160 beetles per day. The difference is significant (p-value = 0.022; Welch Two Sample t-test). Thus, barrel traps equipped with UVLEDs caught 54% more beetles tan those without UVLEDs.

Figure 1: CRB barrel trap.

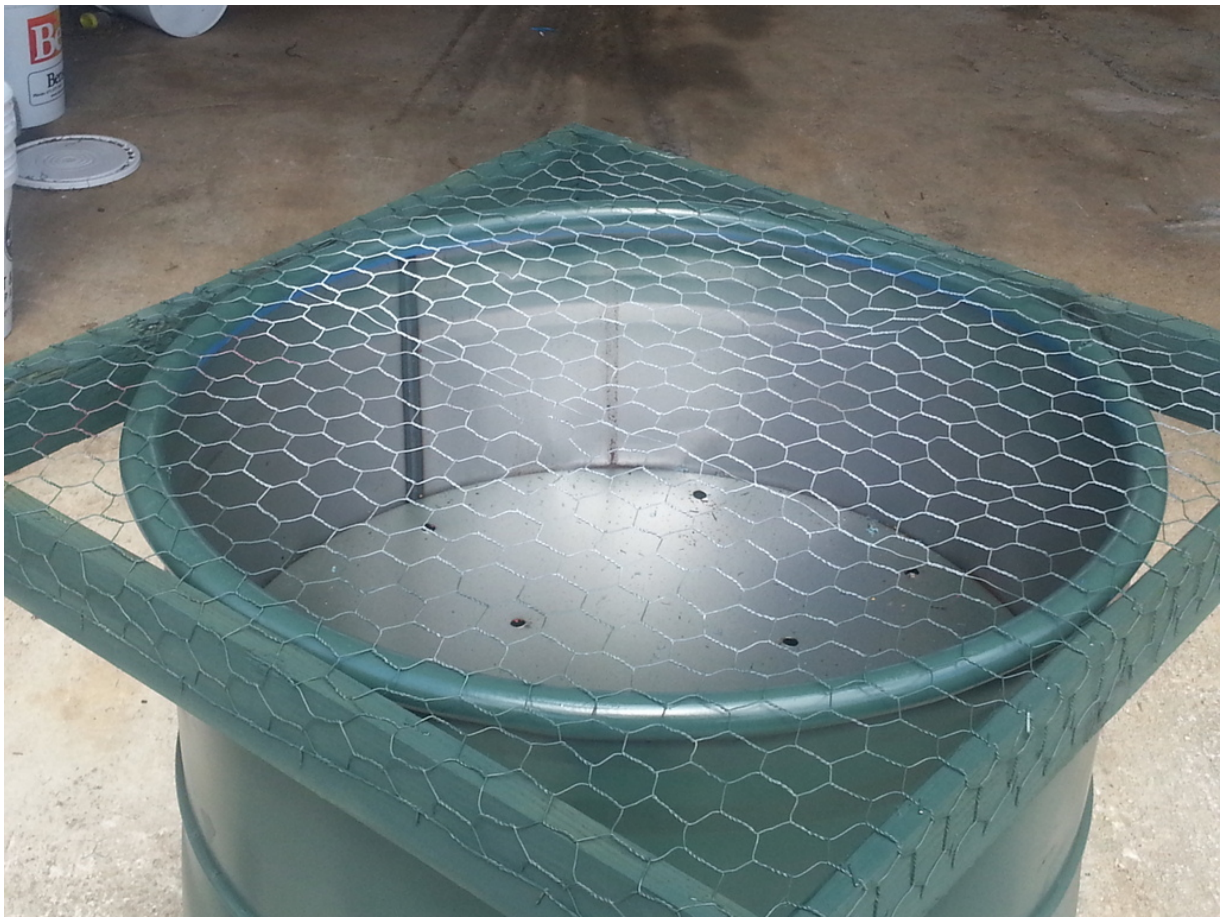
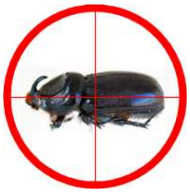


Figure 2: CRB barrel trap fitted with a pan to facilitate counting newly arrived adults.

8 Appendix: 2013-11-06A Improved Pheromone Traps for Coconut Rhinoceros Beetle

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Improved Pheromone Traps for Coconut Rhinoceros Beetle

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

November 6, 2013*

A field trial was conducted to test increased attractiveness of standard CRB pheromone traps by addition of ultraviolet light emmitting diodes (UVLEDs) and use of reduced release rate lures.

UVLEDs increased the trap catch rate by almost 3X when used in conjunction with pheromone lures. Only 2 CRB were caught in traps equipped with a UVLED but without a pheromone lure, indicating that the light sources act synergistically with pheromone lures. Our use of inexpensive solar powered UVLEDs is novel.

There was no significant difference in trap catch rate between traps equipped with standard and reduced release rate lures, even though th release rate was reduced by an average of 90%.

1 Methods

1.1 Traps

Linear trap lines, each with six traps, were established at six locations on Guam. Trap lines were set perpendicular to prevailing winds and the distance between adjacent traps was 20 to 50 m.

*Revised November 6, 2013
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/improvedPheromoneTraps

Standard CRB pheromone traps ([1]) were suspended at 3 m above the ground from forked sticks. We tested six trap treatrments at each location:

T: standard vaned-baffle bucket trap

T+SL: trap + standard lure

T+RL: trap + reduced release rate lure

T+UV: trap + UVLED

T+SL+UV: trap + standard lure + UVLED

T+RL+UV: trap + reduced release rate lure + UVLED

Traps were visited biweekly over a period of twelve weeks. During each trap visit pheromone lures were replaced and trapped CRB were counted and sexed. Treatments were assigned to traps using a randomization scheme which placed all treatments once at each trap site during the experiment.

1.2 Pheromone Lures

We used Oryctalure manufactured by Chemtica. These lures are bubble packs which use a plastic membrane to regulate the release rate of the CRB aggregation pheromone (ethyl 4-methyloctenate). In this experiment, we weighed lures before deployment and after pick up so that we could measure field release rates. Preliminary work showed that rain water entered Oryctalures making it impossible to accurately measure release rates. To solve this problem, we heat-sealed each Oryctalure into a thin polyethylene bag, reducing the release rate by about 10%. We made reduced-release rate lures by placing 200 microlitres of liquid removed from an Oryctalure into a 2 ml Eppendorf centrifuge tube with a 2 mm (5/64 inch) hole drilled in its top. The centrifuge tube was then placed in a pottle which acted as a rain and wind shield (Figure 1).

1.3 Ultraviolet Light Emitting Diodes

We attached two types ultraviolet light emitting diode (UVLED) devices to the baffles on our traps.

Type 1: The original prototype, manufactured by collaborators at USDA-ARS-PBARC, used a battery pack of eight AA batteries to power 4 UVLEDs. We added a 1 k ohm resistor to reduce current from 5.8 to 1.0 ma.with no apparent reduction in brightness. Thus the increasing battery life by at least 5 times..

Type 2: We converted solar powered lawn path lights by replacing the standard white LED with a single UVLED which had been sanded to make it diffuse and omnidirectional.

2 Results and Discussion

2.1 Release Rates

Mean release rates for the standard and reduced rate lures were 14.32 mg/day and 1.41 mg/day, respectively (p ¡ 2E-16; t-test)(Figure 2).

Figure 1: Reduced release rate lure.

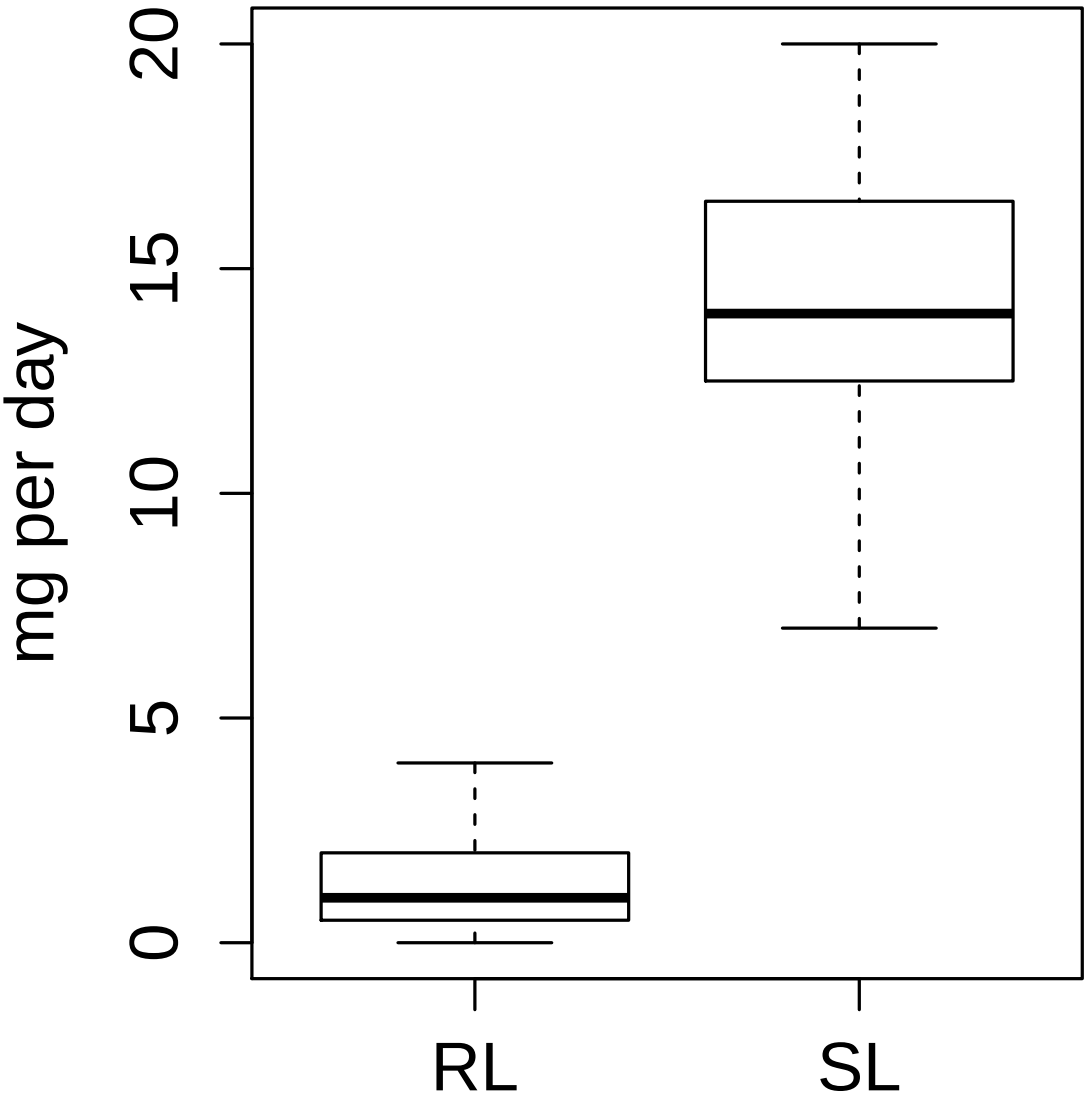


Figure 2: Release rates for standard and reduced rate lures.

2.2 Trap Catch

Statistical analysis of data from this experiment is still preliminary and conclusions may change prior to publication. However, here is what analysis indicates to date:

- Traps equipped with a pheromone lure and UVLED had a significantly higher trap rate than those without a UVLED: 0.091 versus 0.033 beetles per trap-day, respectively ($p = 0.008$; t-test).
- Difference in trap rate between standard rate lures and reduced rate lures was insignificant: 0.074 versus 0.050 beetles per trap-day, respectively ($p = 0.291$; t-test).
- All traps equipped with pheromone lures trapped approximately equal numbers of males and females: 68 versus 57 beetles, respectively ($p = 0.371$; binomial test for equal proportions).

References

[1] Rebecca H Hallett, A L Perez, G Gries, R Gries, Jr H. D. Pierce, Junming Yue, A C Oehlschlager, L M Gonzales, and John H. Borden. Hallett 1995 aggregation pheromone coconut rhinoceros beetle oryctes.pdf. pages 1549–1570, 1995.

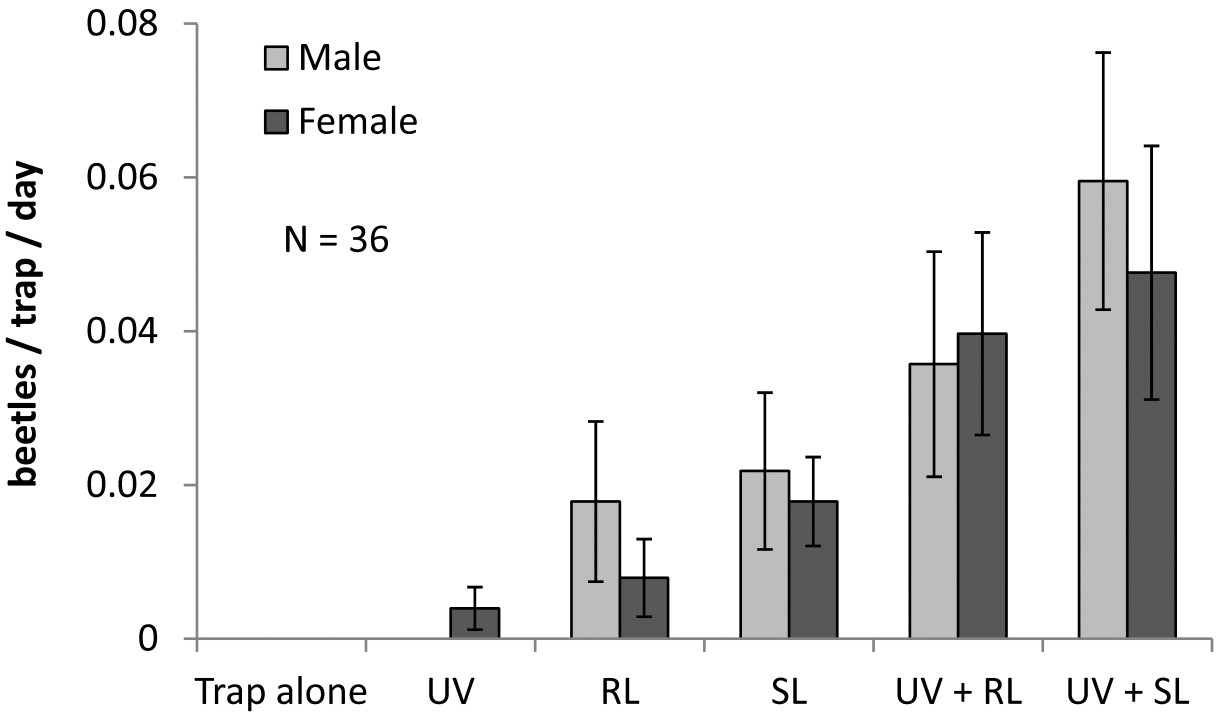
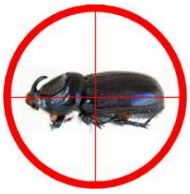


Figure 3: Mean daily trap catch for each trap type.

9 Appendix: 2014-01-08 Relative Attractiveness of White and Ultraviolet Light Emitting Diodes Plus Oryctalure

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



DRAFT: Relative Attractiveness of White and Ultraviolet Light Emitting Diodes Plus Oryctalure

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

January 8, 2014*

Abstract to appear here any day now.

1 Methods

We measured the attractiveness of white light emitting diodes (LEDs) versus ultraviolet LEDs in a series of A-B selection experiments performed in two large field cages at the University of Guam Agricultural Experiment Station at Yigo on the evenings of January 2 through 7, 2014 See figure 1for the experimental setup. We tested three types of LEDs: a white LED (W) and two ultraviolet LEDs. To human eyes, one type had a blue color (B) and the other had a violet color (V). In each cage, we ran all permutations (WB, WV, BV, BW, VW, VB) in random order on consecutive nights (Table 1). Test beetles came from pheromone traps.

*Revised January 9, 2014
C:/Documents and Settings/Administrator/My Documents/LEDcolor/LEDcolor.lyx

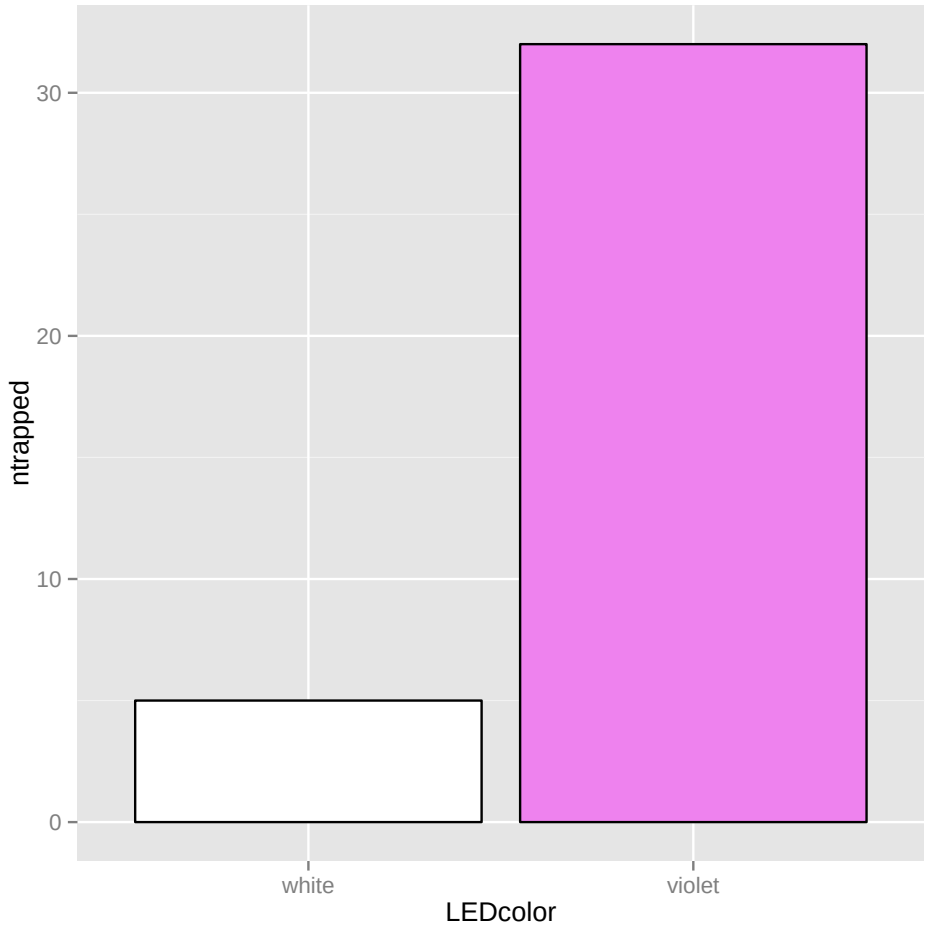
Figure 1: Experimental setup. Release site (CRB adults in peat moss) in foreground. Two barrel traps equipped with pans and LEDs in background. A single oryctalure was hung between the barrels. The experiment was performed in two large field cages (20' x 20' x 10').

2 Results and Discussion

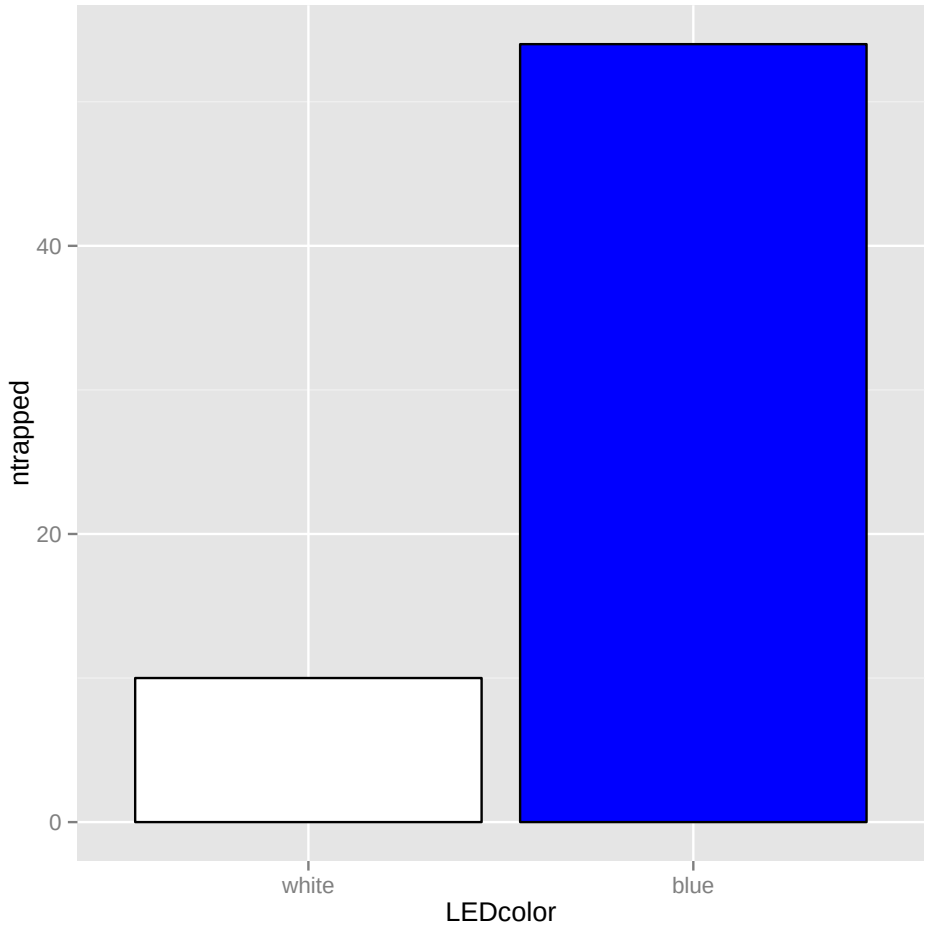
Both types of UVLEDs, when used in conjunction with oryctalure, resulted in trap catches significantly higher than those obtained with white LEDs.There was no difference in trap catch obtained using the different types of UVLEDs.

Table 1: Raw data.

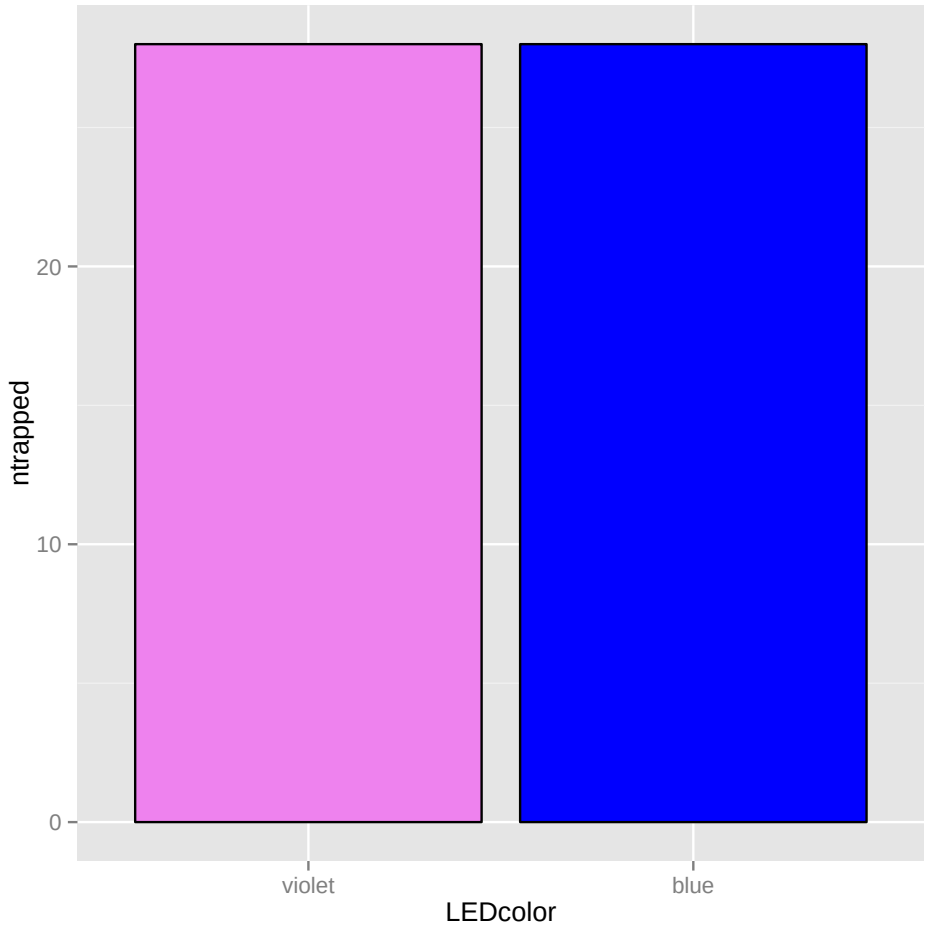
	Day	Cage	Permutation	LeftCount	RightCount	UntrappedCount
1	1	N	VW	9	1	8
2	1	S	BW	11	2	5
3	2	N	BV	8	12	7
4	2	S	VW	13	2	6
5	3	N	BW	8	1	4
6	3	S	BV	6	7	6
7	4	N	WV	1	3	9
8	4	S	WV	1	7	12
9	5	N	WB	3	13	26
10	5	S	WB	4	22	10
11	6	N	VB	7	4	17
12	6	S	VB	2	10	6




```
> binom.test(c(white,violet))

        Exact binomial test

data:  c(white, violet)
number of successes = 5, number of trials = 37, p-value = 7.428e-06
alternative hypothesis: true probability of success is not equal to 0.5
95 percent confidence interval:
 0.04537199 0.28774780
sample estimates:
probability of success
             0.1351351
```



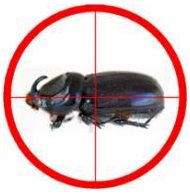

```
> binom.test(c(white,blue))

        Exact binomial test

data:  c(white, blue)
number of successes = 10, number of trials = 64, p-value = 1.996e-08
alternative hypothesis: true probability of success is not equal to 0.5
95 percent confidence interval:
 0.07755733 0.26863424
sample estimates:
probability of success
               0.15625
```




```
> binom.test(c(violet,blue))

        Exact binomial test

data:  c(violet, blue)
number of successes = 28, number of trials = 56, p-value = 1
alternative hypothesis: true probability of success is not equal to 0.5
95 percent confidence interval:
 0.3633554 0.6366446
sample estimates:
probability of success
                   0.5
```

10 Appendix: 2014-01-12A Chicken Wire Escape Test

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Chicken Wire Escape Test

Prepared by
Aubrey Moore and Roland Quitugua
University of Guam Cooperative Extension Service

January 17, 2014*

Escape rates were 92% and 28% for the uncovered pan and the chicken wire covered pan, respectively.

1 Methods

At 6:30 PM on January 12, 2014, two pan traps without UVLEDS, lures breeding materilawere set up next to each other in a large field cage. 29 beetles were placed in left-hand pan and this was covered with chicken wire. 25 beetles were placed in the right hand trap and this was left uncovered. Beetles remaining in pans were counted at 9:30 PM.

2 Results and Discussion

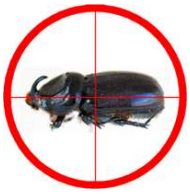
21 of 29 beetles remained in the pan capped with chicken wire. 2 of 25 beetles remained in the uncovered pan. Escape rates were 92% and 28% for the uncovered pan and the chicken wire covered pan, respectively.

*Revised February 10, 2014
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/2014-0-12A Chicken Wire Escape Test/CRB2014-01-12A.lyx

Figure 1: Experimental setup.

11 Appendix: 2014-01-12B Plastic Top Catch Test

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Plastic Top Catch Test

Prepared by
Aubrey Moore and Roland Quitugua
University of Guam Cooperative Extension Service

January 17, 2014*

Pans covered with chicken wire and plastic top both caught beetles. There was no significant difference in trap catch.

1 Methods

Two pan traps were set up next to each other in a large field cage, one covered with chicken wire, the other covered with a plastic top with XX inch holes. A single oryctalure was hung between the traps (Fig. 1). Beetles were released downwind at 6:30 PM and trap catch was counted at 9:30 PM.

2 Results and Discussion

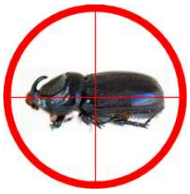
Two beetles were caught in the pan with the plastic cover and 2 more were walking on the cover. The metal pan contained 3 beetles. A total of 7 beetles were found at large in the cage.

*Revised February 10, 2014
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/2014-01-12B Plastic Top Catch Test/CRB2014-01-12B.lyx

Figure 1: Experimental setup.

12 Appendix: 2014-01-15 Chicken Wire vs Plastic Top

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Chicken Wire vs Plastic Top

Prepared by
Aubrey Moore and Roland Quitugua
University of Guam Cooperative Extension Service

January 17, 2014*

Escape rate from a pan covered with chicken wire was 24%. Escape rate from a pan covered with a plastic top with holes was 0%.

1 Methods

A metal pan with chicken wire cover and a plastic pan with a plastic cover with XX holes were placed on the floor of a large field cage (Fig. 1). Sixty-two beetles were placed in each pan at 6:30 PM on January 15, 2014. Remaining beetles wer counted at 9:30 PM.

2 Results and Discussion

Forty-seven beetles remained in the metal pan covered with chicken wire (escape rate = 24%) and all 62 beetles remained in the plastic pan covered with the plastic lid with holes (escape rate = 0%).

Two beetle escapes through the chicken wire were obsered directly. In the first case, the beetle flew up the side of the metal pan and got its head through one of th holes in th wire. In the second case, the a flying beetle poked its head through a hole near the center of th chicken wire. It was

*Revised February 10, 2014
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/2014-01-15 Chicken Wire vs Plastic Top\CRB2014-01-15.lyx

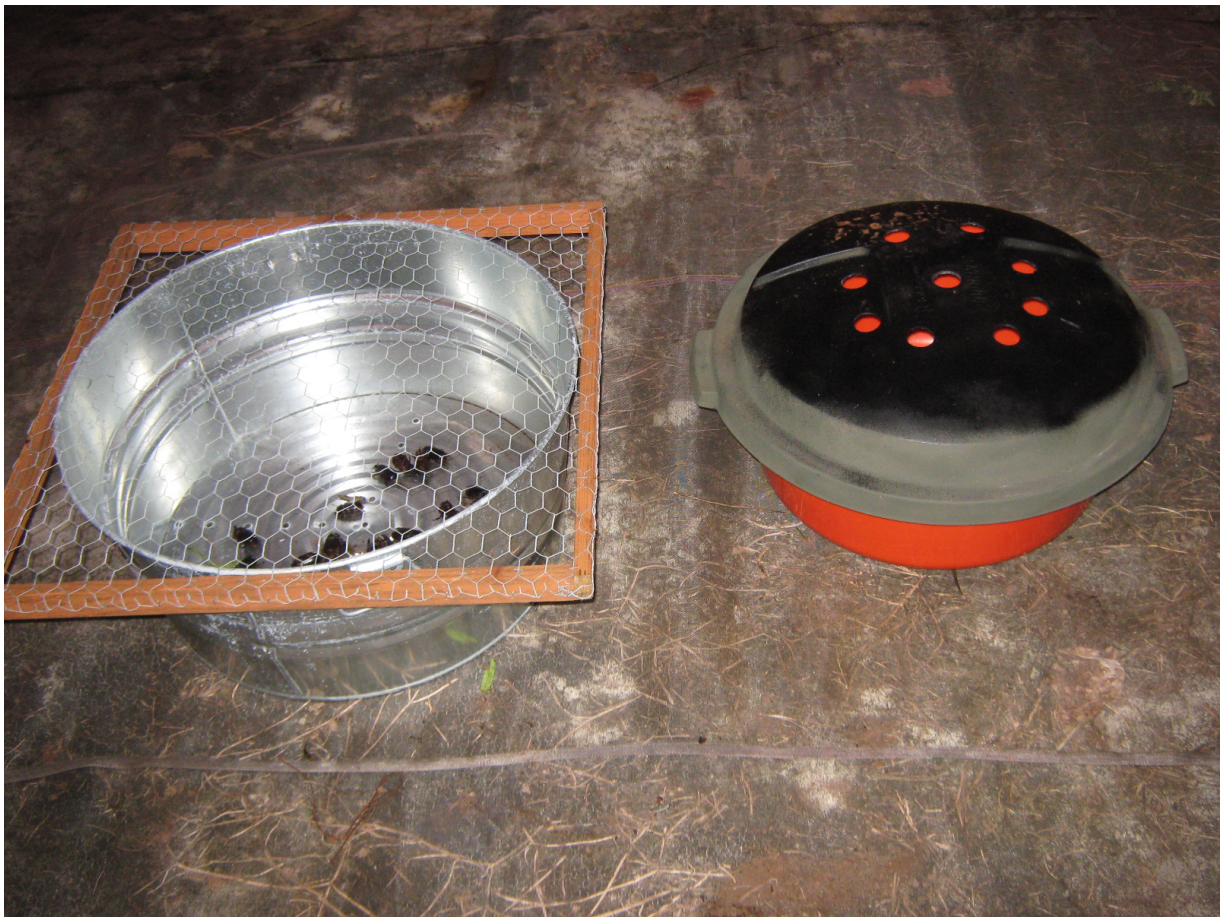


Figure 1: Experimental setup.

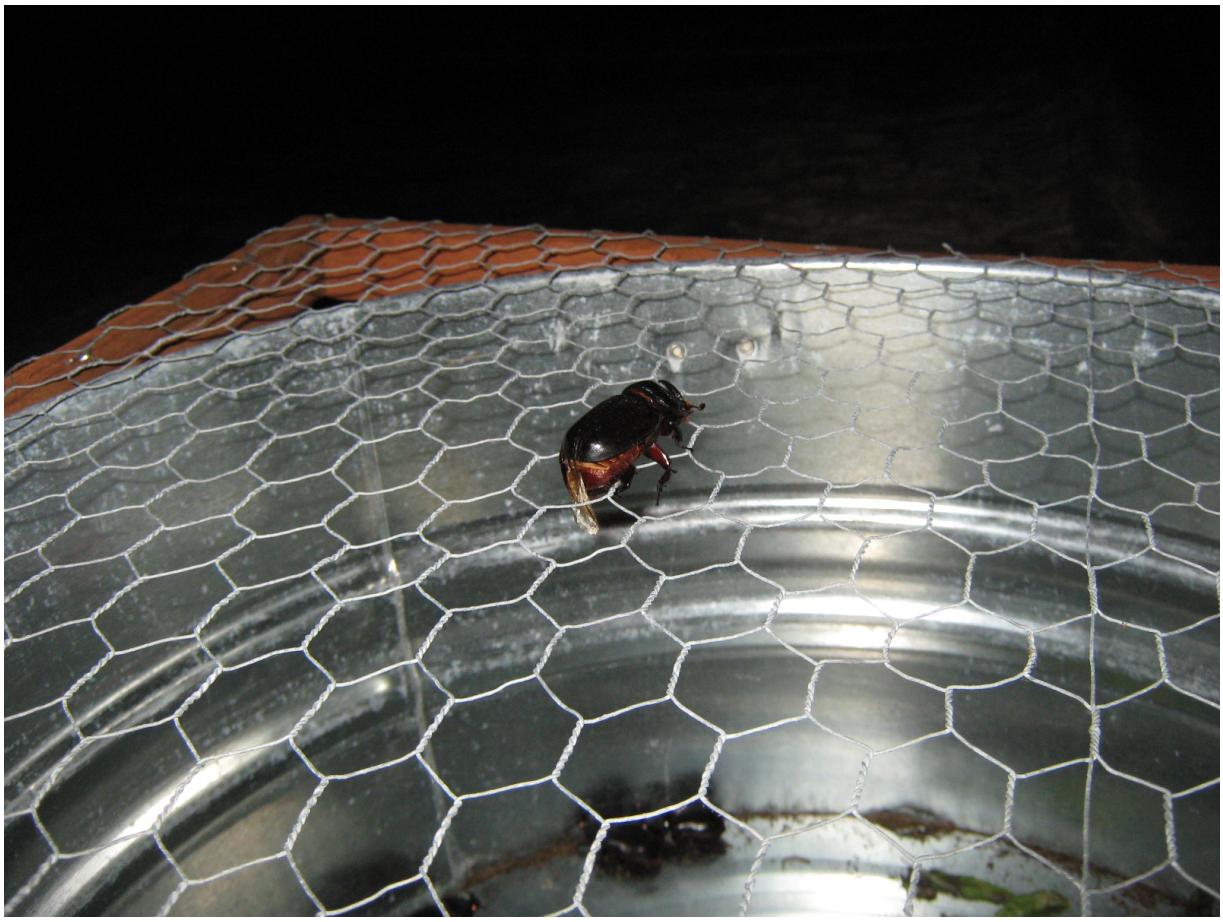
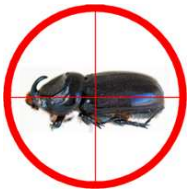


Figure 2: Escaping beetle.

able hang on with its legs, and close its elytra before crawling out on top of the chicken wire (Fig. 2).

13 Appendix: 2014-01-16 Minibucket Test

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Minibucket Test

Prepared by
Aubrey Moore and Roland Quitugua
University of Guam Cooperative Extension Service

January 17, 2014*

This trap design catches beetles.

This is a test of a trap designed so that it can be built with mostly inexpensive items which can be purchased at most hardware stores. The UVLED and oryctalure must be provided separately.

1 Methods

A small paint bucket was placed in a cutout in the top of a plastic garbage can (Fig. 1). The top of the bucket contained four one-inch diameter holes. A UVLED was fitted through a hole at the center of the lid. An oryctalure was hung from the bottom of the UVLED inside the bucket. The larger garbage can was empty.

The experiment was performed in a large field cage. Beetles were released about 16 feet downwind of the trap at 7:00 PM on January 16, 2014. Beetle response to the trap was observed visually and also using a time lapse infrared camera programmed to take an image every five seconds. The experiment was closed down and trapped beetles were removed from the trap at 9:30 PM.

*Revised February 10, 2014
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/2014-01-16 Minibucket Test/CRB2014-01-16.lyx

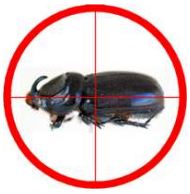
Figure 1: Experimental setup.

2 Results and Discussion

Four beetles were trapped and 18 beetles were collected 'at large' within the cage. Two beetles were observed entering the trap. Both entered abdomen first. A time lapse video of the experiment is available at `http://www.youtube.com/watch?v=nvE6pJ6Q3FY&feature=youtu.be`.

14 Appendix: 2014-01-17 Minibucket Escape Test

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Minibucket Escape Test

Prepared by
Aubrey Moore and Roland Quitugua
University of Guam Cooperative Extension Service

January 17, 2014*

None of 48 beetles escaped from this trap.

1 Methods

A small paint bucket was placed in a cutout in the top of a plastic garbage can (Fig. 1). The top of the bucket contained four one-inch diameter holes. A UVLED was fitted through a hole at the center of the lid. An oryctalure was hung from the bottom of the UVLED inside the bucket. The larger garbage can was empty.

The experiment was performed in a large field cage. Forty-eight beetles were placed in the trap at 6:00 PM on January 17, 2014. Beetles remaining in the trap were counted th following morning.

2 Results and Discussion

All 48 beetles remained in the trap.

*Revised February 10, 2014
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/2014-01-17 Minibucket Escape Test/CRB2014-01-17.lyx

Figure 1: Experimental setup.

15 Appendix: 2014-01-17A Hawaii Beetle Dissections

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project

Hawaii Beetle Dissections

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service
and
Sean Marshall
AgResearch, New Zealand

January 17, 2014*

Four beetles from Hawaii were sexed, measured and dissected to look for eggs. Sample 1 was an interception in the arrivals baggage area of the Honolulu International Airport. The remainging three specimens were from pheromone trap CRB4 deployed on a golf course at the Hickam Air Force Base. All were female; two were gravid but we did not find embryos.

1 Notes

20140114.001 (interception # APWJI????; International airport on 2013/??/?? baggage carousel)

Female
Body length: 49 mm (L)
Elytra dimensions: 27 x 21 mm (LxW)

*Revised February 12, 2014
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/2014-01-17A Hawaii Beetle Dissections/CRB2014-01-17A.lyx

Dissection: many eggs easily observed, no obvious embryo within the 1 egg that was dissected (1, 2, 3).

20140114.002 (interception # APWHI133641003001; trap#CRB4 on 2013/12/30 near airforce base)

Female
Body length: 44mm (L)
Elytra dimensions: 25 x 19 mm (LxW)
Dissection: initial tissue appeared to be undergoing initial putrification, no obvious eggs seen (4).

20140114.003 (interception # APWHI140021003001; trap#CRB4 on 2014/01/02 near airforce base)

Female
Body length: 44mm (L)
Elytra dimensions: 27 x 21 mm (LxW)
Dissection: many eggs easily observed, no obvious embryo within the 1 egg that was dissected (5).

20140114.004 (interception # APWHI140021003001; trap#CRB4 on 2014/01/02 near airforce base)

Female
Body length: 42mm (L)
Elytra dimensions: 25 x 19 mm (LxW)
Dissection: initial tissue appeared to be undergoing initial putrification, no obvious eggs seen (6).

2 Ackowledgments

Thanks to USDA-APHIS-PPQ for releasing the Hawaii CRB specimens to us.

This work was done by AgResearch New Zealand in collaboration with the University of Guam with finacial support from a USDA-APHIS grant.

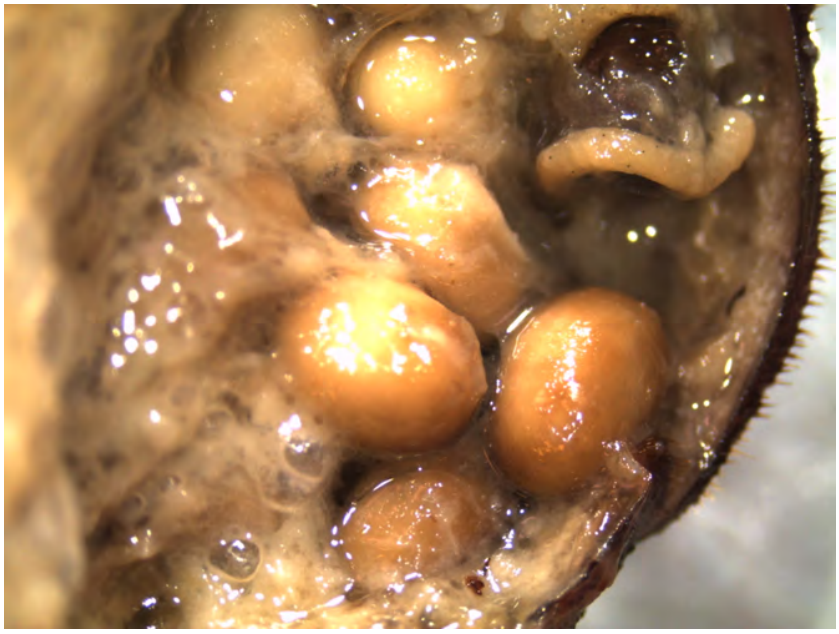


Figure 1: Eggs in beetle 20140114.01

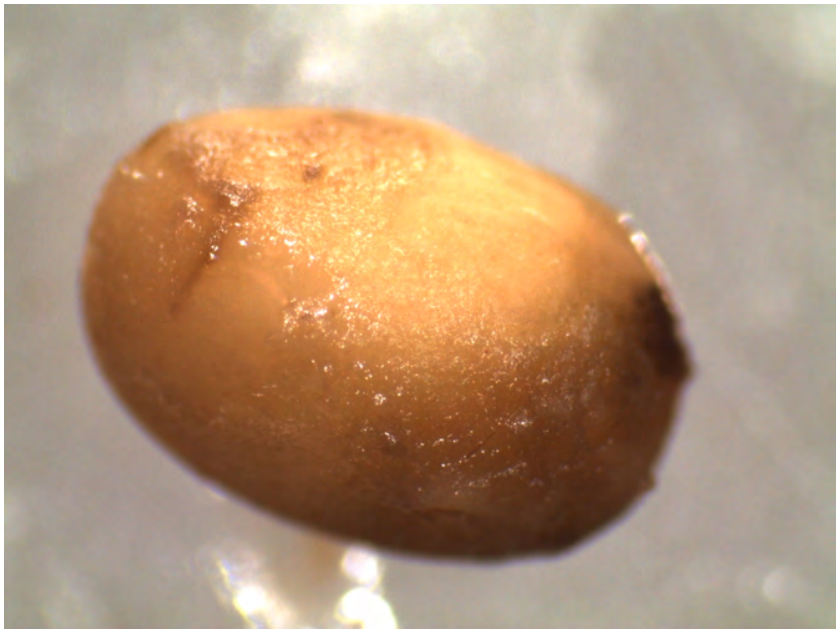


Figure 2: Egg from beetle 20140114.01

Figure 3: No obvious embryo in egg from beetle 20140114.01

Figure 4: Beetle 200140114.02.

Figure 5: Eggs in beetle 200140114.03.

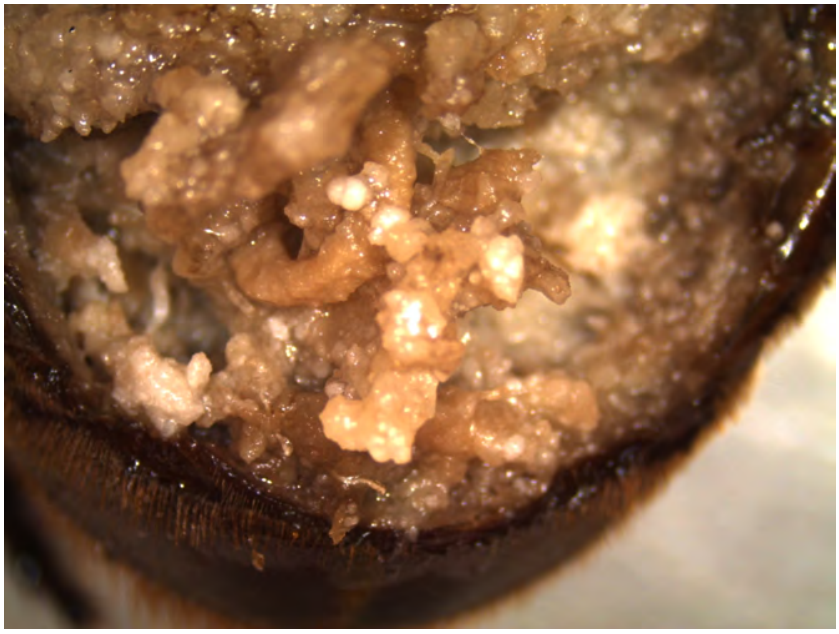
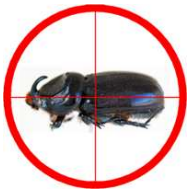


Figure 6: Beetle 200140114.04.

16 Appendix: 2014-02-12 DNA Analysis of Hawaii CRB

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



DNA Analysis of Hawaii CRB

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service
and
Sean Marshall
AgResearch New Zealand

February 12, 2014*

DNA from four CRB adults collected in Hawai were compared to DNA samples from other CRB populations in the Pacidic using RFLP analysis. The Guam and Hawaii populations have DNA which breaks into 253 bp fragments. DNA fragments of this size are absent in DNA samples from Diego Garcia, Fiji, Samoa, and PNG. Thus the Hawaii population may have originated from Guam or a currently unknown common source.

1 Notes

DNA was harvested from hind femurs of CRB adults from Hawaii, Diego Garcia, and Guam and processed to find restriction fragment length polymorphism (RFLP). Collection data for the four Hawaii specimens are provided in a previous technical report. The RFLP results from the Hawaii samples match those from Guam. The results from Diego Garcia matched those from Fiji, Samoa, and Papua New Guinea.

See attachment3 for an image of the RFLP gels.

*Revised February 12, 2014
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/2014-01-17A Hawaii Beetle Dissections/CRB2014-01-17A.lyx

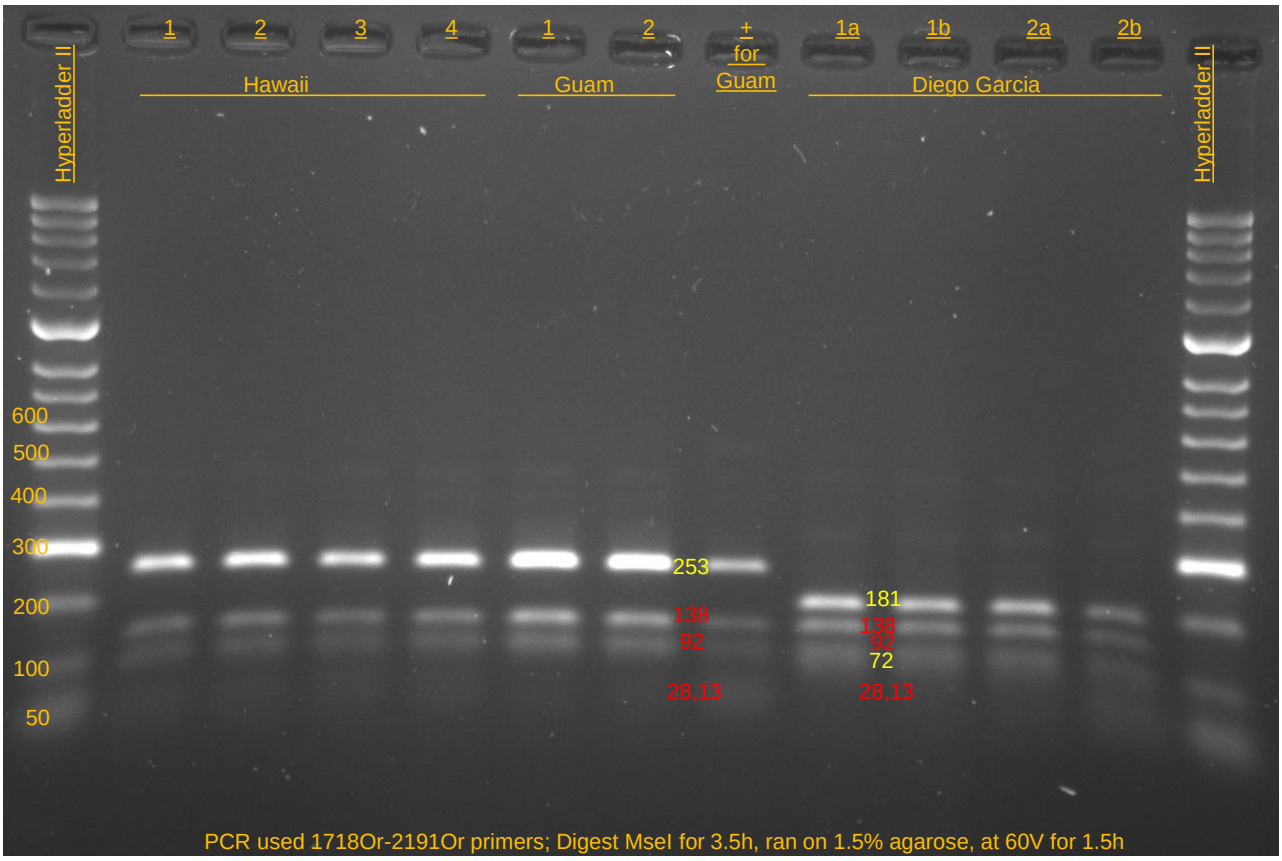
2 Ackowledgments

Thanks to Dan Vice USDA-APHIS Wildlife Services for collecting CRB adults on Diego Garcia on a recent trip. Thanks to USDA-APHIS-PPQ for releasing the Hawaii CRB specimens to us.

This work was done by AgResearch New Zealand in collaboration with the University of Guam with finacial support from a USDA-APHIS grant.

3 Attachment

RFLP of Oryctes rhinoceros COI (1718-2191 amplicon)
-2014/02/112 (gel 634.tif
-cut with MseI to distinguish Guam CRB populations
from other CRB populations (253bp band)



RFLP of Oryctes COI (1718-2191 amplicon)
-2012/09/18 gel 154.tif
-cut with MseI to distinguish
Guam from ‘Fiji/common’ populations