Annual Accomplishment Report USDA-APHIS Farm Bill Grant Guam Coconut Rhinoceros Beetle Eradication Project (Agreement 12-8515-1123-CA)

Prepared by Roland Quitugua and Aubrey Moore
University of Guam

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At a Glance: Recent Progress

- Chemical Control
  - 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.
- 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.
- Other Eradication Project Support
  - We recently developed a new extension flyer on CRB management and held public workshops for pest control professionals and the public.

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 10 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 Appendix: Field Cage Experiment: SPLAT With and Without Cypermetherin 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. Trevor Jackson, AgResearch, New Zealand.

Percent Completion: 15%

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 Appendix: Bioassay of Virus from Fiji for experimental details.

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 Appendix: Field Cage Experiment: Escape Test 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 Appendix: Field Cage Experiment: New Lure vs Depleted Lure 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 Appendix: Reduced Release Rate Lures and Appendix: Improved Pheromone Traps for Coconut Rhinoceros Beetle 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 Appendix: Development of Barrel Traps 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).

- 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.

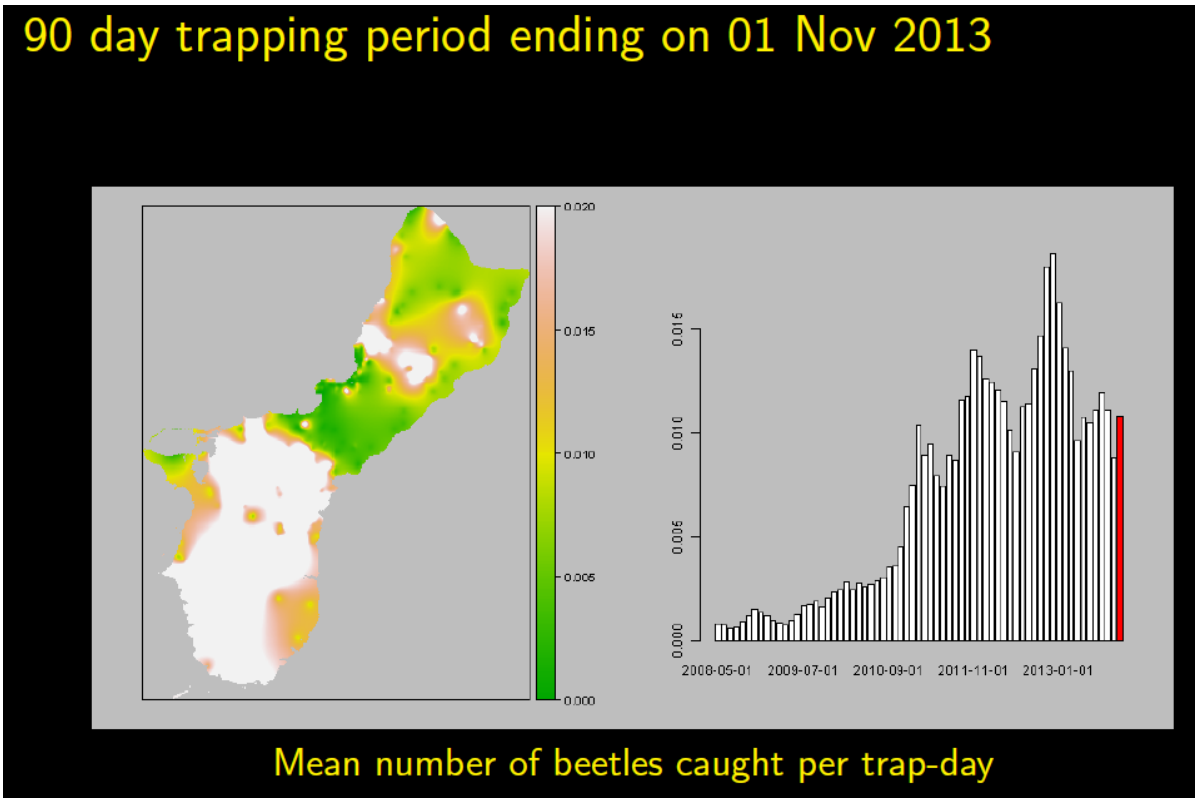
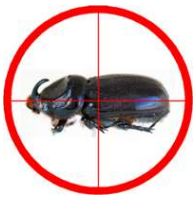


Figure 1: Spatial-temporal display of coconut rhinoceros trap data. This is the last frame from a time series. The entire series can be viewed at http://guaminsects.net/anr/content/visualization-coconut-rhinoceros-beetle-trap-catch-data.

5 Appendix: Bioassay of Virus from Fiji

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



DRAFT
Preliminary Bioassay of Virus from Fiji

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

September 6, 2012

Although this bioassay is not complete, there is indication that Guam's rhino beetles are not susceptible to this virus sample. There are no significant differences in weight loss or mortality between beetles dosed with virus and thos dosed with water.

1 Introduction

This is a preliminary test of virus extracted from diseased CRB guts in Fiji. Two, 2 ml aliquots of this virus were given to Russ Campbell for hand-carry back to Guam by Sean Marshall at the end of the Pacific Plant Protection Organization in June, 2012.

2 Methods

1. One hundred field collected rhino beetles where weighed, sexed and presented with a slice of banana on July 25, 2012. After 24 hours, the beetles where weighed again to determine which had fed.

C:/Documents and Settings/Administrator/My Documents/CRB Virus Bioassay/newStuff

2. Beetles which had gained weight between July 25 and 26, indicating that they had fed, were selected for the virus bioassay. On July 30, sixteen beetles were dosed with 100 microlitres of virus suspension applied to a banana slice. Thirteen beetles were similarly dosed with 100 microlitres of water as an experimental control group.

3. Beetles were weighed and checked for mortality weekly after being dosed. Survivors were fed banana slices. Dead beetles were frozen for subsequent post mortem examination.

4. Throughout the bioassay, beetles were housed individually in 1 pint Mason jars half filled with a commercial steer manure and soil blend. They were kept at in an air-conditioned room at about 24°C.

3 Results

3.1 Dosing

Out of 100 beetles presented with a banana slice on July 25, only 29 showed a positive change in mass on July 26, indicating that they had fed. These 29 beetles were used in the experiment. They were weighed and dosed on July 30 and weighed again 24 h later (Table 1). We rejected 9 beetles which did not feed well on the dosed banana slices, those that gained less than 100 mg, from subsequent analysis.

3.2 Weight Loss

A symptom of virus infection is cessation of feeding. There is no significant difference in weight loss between surviving beetles dosed with virus and those dosed with water (Fig. 1, Fig. 2).

3.3 Mortality

There is no significant difference in mortality between beetles dosed with virus and those dosed with water. To date, two beetles dosed with virus have died and two beetles dosed with water have died (Fig. 2).

Table 1: Change in mass during 24h when beetles were presented with dosed banana slices.

	Beetle	Change in mass(mg)
1	883	-78
2	1850	-76
3	1856	-56
4	1867	-6
5	1877	-3
6	1890	44
7	703	56
8	1869	64
9	510	83
10	714	125
11	1894	179
12	699	250
13	1870	295
14	1861	299
15	1857	302
16	1855	318
17	1878	370
18	435	448
19	1863	459
20	696	533
21	1889	608
22	718	621
23	1874	687
24	570	718
25	1866	725
26	527	813
27	1626	816
28	1624	946
29	1628	1016

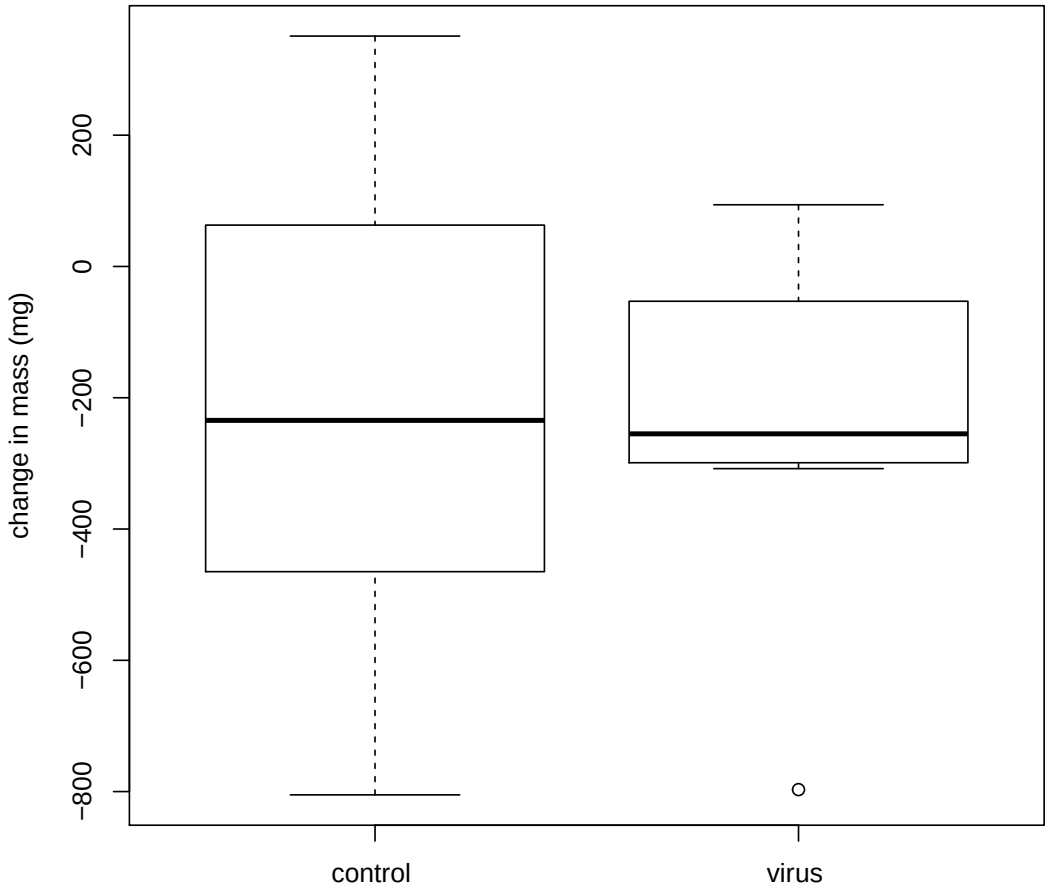


Figure 1: Change in mass of survivors during the period between when they were dosed (2012-07-31 09:20:00) and when they were last weighed (2012-09-04 09:00:00). P-value equals 0.8934.

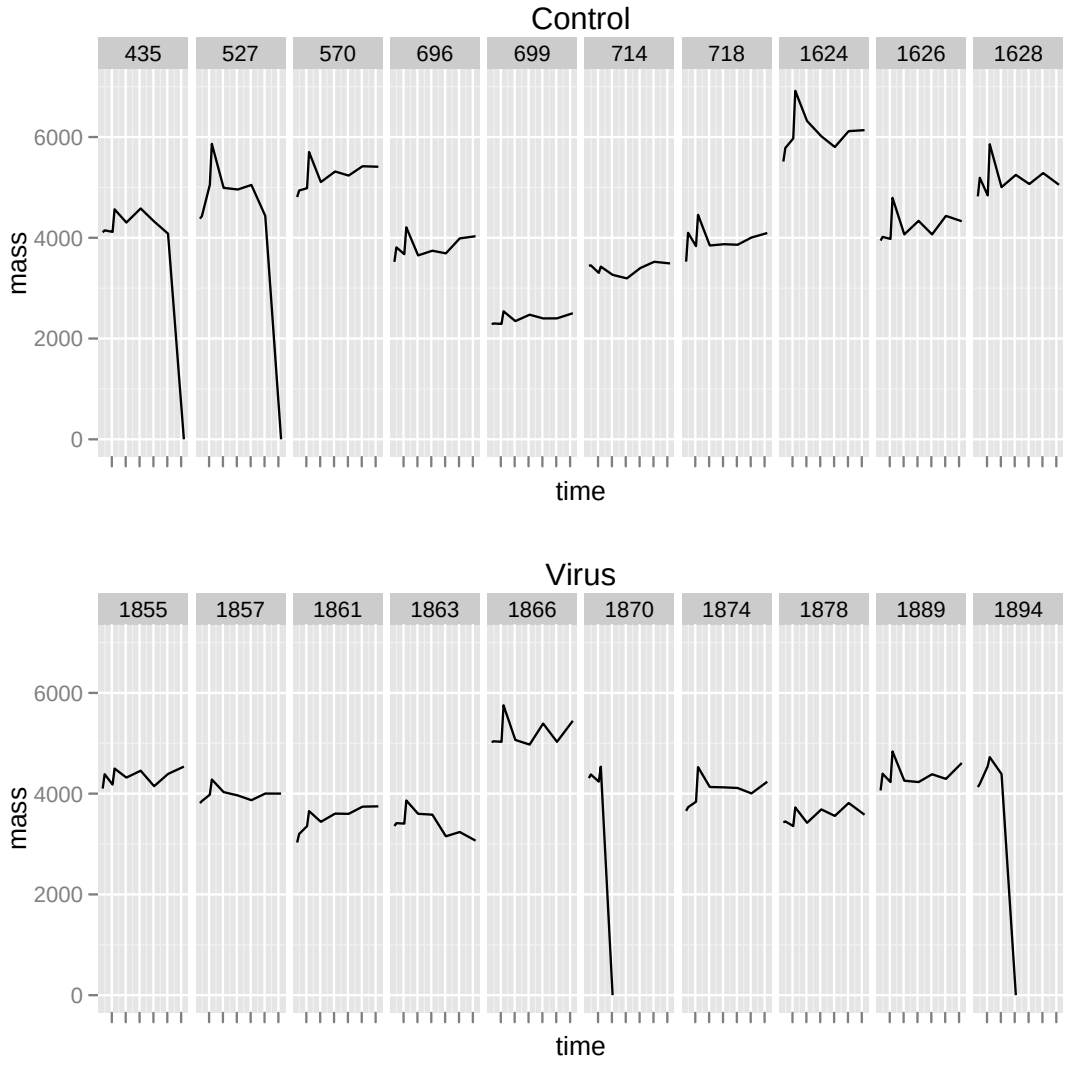
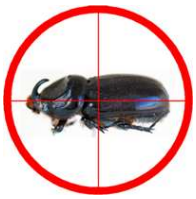


Figure 2: Change in mass of survivors during the period between when they were dosed (2012-07-31 09:20:00) and when they were last weighed (2012-09-04 09:00:00). Death of a beetle is indicated by a mass of zero.

6 Appendix: Field Cage Experiment: New Lure vs Depleted Lure

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Field Cage Experiment
New Lure vs Depleted Lure

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

September 23, 2012*

We performed a semifield experiment in which coconut rhinoceros beetles were allowed to fly freely in two large field cage (20' x 20' x 10'). In one cage, we placed a single baffled bucket trap baited with a new Oryctalure®and in the other cage we placed an identical trap baited with a depleted lure. The experiment was replicated once. Traps caught between 62% to 78% of flying beetles. Difference in trap-catch between traps baited with new lures and depleted lures were not significant. Estimated pheromone release rate is 17.0 mg per day for the new lure and 0.4 mg per day for the depleted lure.

It is possible that the Oryctalure release rate is too high and that beetles are becoming arrested or repelled as they approach traps baited with fresh lures. Guam trapping records show that, on average, more than twice the number of beetles was caught in depleted traps than in traps with new lures and this is statistically very highly significant.

*Revised March 29, 2013
C:/Documents and Settings/Administrator/My Documents/CRB Field Cage/depleted

1 Introduction

Mass trapping was ineffective in protecting mature coconut palms on Guam. In Guam's Tumon Bay area severe defoliation has been experienced within high density trapping areas in the hotel landscaping environment. In the current experiment, we measured trap efficacy for beetles flying within large field cages and compared trap-catch in a trap with fresh lure, and one baited with a depleted lure.

2 Methods

2.1 Beetles

For each experiment, we field collected adult coconut rhinoceros beetles, *Oryctes rhinoceros*. These were housed in two plastic tubs half filled with peat moss, 30 beetles in each tub. The beetles were fed bananas two days prio to the start of each experiment. In experiment 1, beetles were fed a second time, during the experiment, on May 17. Beetles were kept in an air conditioned room when not being used in flight tests.

2.2 Field Cages

Experiments were performed in two custom-designed large field cages (20' x 20' x 10') erected at the University of Guam's Agricultural Experiment Station in Yigo (Fig. 1).

2.3 Traps and Lures

We used standard traps and lures (Oryctalure®, ChemTica, Costa Rica) used by the Guam Coconut Coconut Rhinoceros Beetle Eradication Project (Fig. 2). Each lure consists of a liquid rhino beetle aggregation pheromone contained in a clear plastic membrane. The pheromone is colored red which makes it easy to determine how much liquid is left in the lure. Traps are baffled bucket traps made locally. Baffles are made out of Coroplast®and the buckets are standard seven gallon paint buckets. A lure is hung in a hole cut at the center of the baffle. In one cage, we placed a single baffled bucket trap baited with a new Oryctalure®and in the other cage we placed an identical trap baited with a depleted lure. No liquid was evident in the depleted lure. We estimated the release rate of pheromones from lures used in the second experiment by wieghing the lures at 900h on June 7, hanging both lures in the shade under a canopy, and reweighing the lures at 1300h on June 17.

2.4 Flight Tests

Flight tests were only run during evenings in which the average wind speed was less than 5 mph, as measured by a weather station only 300' from the field cages, and when the probability of rain during the test period was low. At about 30 minutes prior to sunset, a plastic tub containing 30 beetles was put in each cage and the lid was removed. Beetles cannot crawl out of tubs, but they can fly out. Each cage contained a trap hung

Figure 1: Large, custum-designed field cages (20' x 20' x 10') used for semifield experiments with the coonut rhinoceros beetle.

at about 6 feet above the ground and One cage contained a trap baited with a new lure and the other contained a trap baited with a depleted lure.

After making a decision to run a flight test, at about 30 minutes prior to sunset, a tub containing 30 beetles was placed in each cage and the lid was removed. Location of the trap and the tub were adjusted so that the trap was directly upwind with respect to the tub.

At about three hours after sunset, beetles were collected, counted and returned to their tubs. Beetles which had been trapped and those found elsewhere with the cage were tallied.

3 Results

Beetles became active and started emerging from the peat moss in the tubs at sunset. They began to fly at about 15 minutes after sunset and flight activity lasted for about one hour. Direct observations confirmed that beetles were unable to crawl out of the tubs.

In both experiments, the trap in each cage caught about 75% of those which flew (Table 1, Table 2). The trap baited with the deplete lure caught as many flying insects as the trap baited with a new lure(Table 3, Table 4).

Estimated pheromone release rate is 17.02 mg per day for the new lure and 0.39 mg per day for the depleted lure.

Figure 2: Standard veined-baffle bucket trap used by the Guam Coconut Rhinoceros Eradication Project. Note Oryctalure®hung at the center of the baffle.

Table 1: Experiment 1 data summary.

	Date	Cage	Beetles	Lure	Flyers	Trapped
1	05/15/12	N	C2	Dep	9	8
2	05/15/12	S	C1	New	5	4
3	05/16/12	S	C2	New	5	4
4	05/18/12	N	C2	Dep	2	2
5	05/18/12	S	C1	New	8	6
6	05/19/12	N	C1	Dep	4	3
7	05/19/12	S	C2	New	5	4
8	05/25/12	N	C2	New	4	3
9	05/25/12	S	C1	New	1	1
10	05/26/12	N	C1	New	4	2
11	05/26/12	S	C2	Dep	2	0
12	05/27/12	N	C2	New	1	1
13	05/27/12	S	C1	Dep	4	2
14	05/28/12	N	C1	New	0	0
15	05/28/12	S	C2	Dep	2	0
16	05/29/12	N	C2	New	1	0
17	05/29/12	S	C1	Dep	1	0

Table 2: Experiment 2 data summary.

	Date	Cage	Beetles	Lure	Flyers	Trapped
1	06/04/12	N	G2	New	16	12
2	06/04/12	S	G1	Dep	13	11
3	06/05/12	N	G1	New	4	3
4	06/05/12	S	G2	Dep	5	3

Table 3: Experiment 1 results. Difference in proportions of flying beetles trapped by a new lure and a depleted lure are not significant (t-test, $p = 0.5445$).

	Lure	Flyers	Trapped	Proportion trapped
1	Dep	24	15	0.62
2	New	34	25	0.74

Table 4: Experiment 2 results. Difference in proportions of flying beetles trapped by a new lure and a depleted lure are not significant (t-test, p = 1).

	Lure	Flyers	Trapped	Proportion trapped
1	Dep	18	14	0.78
2	New	20	15	0.75

4 Discussion

After discovering that a trap baited with a depleted lure catches an equivalent number of beetles as a trap baited with a new lure, I checked trapping records from operational traps. Trappers hang a new lure in each trap whenever they observe that all the liquid has evaporated from the previously applied Oryctalure controled release dispenser. Thus we can calculate the trap-catch from traps with depleted lures by selecting records from the `trap_visits` table where `lure_replaced` is true:

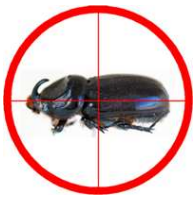
```
# Finds number of beetles caught in traps baited with
# depleted lures (lure_replaced = 'Y') in comparison to those baited
# with undepleted lures (lure_replaced = 'N').
# Trap routes operated by Mary, Grimm, and Wenninger were excluded
# because these records were entered manually and lure replacement
# was often not recorded.
#########################################################################
require(RODBC)

## Loading required package:  RODBC

conn = odbcConnect("oryctes")
sql = paste(
   "SELECT",
   "  lure_replaced,",
   "  (male_count+female_count+unsexed_count) AS trap_catch",
   "FROM trap_visit",
   "WHERE",
   "  lure_replaced NOT LIKE ''",
   "  AND (person_id NOT LIKE '%Mary%')",
   "  AND (person_id NOT LIKE '%Grimm%')",
   "  AND (person_id NOT LIKE '%Wenninger%')")
dat=sqlQuery(conn, sql)
odbcClose(conn)
t.test(trap_catch~lure_replaced, data=dat)

##
##  Welch Two Sample t-test
##
## data:  trap_catch by lure_replaced
## t = -16.33, df = 25492, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.1852 -0.1455
## sample estimates:
```

```
## mean in group N mean in group Y
##          0.1032          0.2686
##
```

As you can see, on average, more than twice the number of beetles was caught in depleted traps than in traps with new lures and this is statistically very highly significant. It is possible that the Oryctalure release rate is too high and that beetles are becoming arrested or repelled as they approach traps baited with fresh lures. Miller et al. 2005, working on dose-dependent pheromone responses of mountain pine beetle found that Lidgren trap catches decreased by about one order of magnitude when the pheromone release rate was increased above an optimum of about 0.5 mg per day.

7 Appendix: Field Cage Experiment: SPLAT With and Without Cypermetherin

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Field Cage Experiment
SPLAT With and Without Cypermethrin

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

September 25, 2012

We performed a semifield experiment in which coconut rhinoceros beetles were allowed to fly freely in two large field cage (20' x 20' x 10'). In one cage, we placed a target smeared with SPLAT-RB®, a sticky matrix containing *Oryctes rhynoceros* aggregation pheromone. In a second cage, we placed a target smeared with an experimental attracticide, SPLAT-RB plus 5% cypermethrin.

1 Introduction

This semi-field experiment tests an experimental attracticide for adult Oryctes rhinoceros beetles, SPLAT-RB®plus 5% cypermethrin, manufactured by ISCA. SPLAT-RB®is a sticky matric containing synthetic *O. rhinoceros* aggregation pheromone. This product is used in conventional traps. Addition of 5% cypermethrin creates an attracticide which can be used without a trap. Three things have to happen for the attracticide to work:

1. A beetle has to be attracted to the target.
2. The beetle has to make physical contact with the target.

C:/Documents and Settings/Administrator/My Documents/CRB Field Cage/splat

3. The beetle has to absorb a lethal dose of cypermethrin.

2 Methods

2.1 Beetles

For each experiment, we field collected adult coconut rhinoceros beetles, *Oryctes rhinoceros*. These were housed in two plastic tubs half filled with peat moss, 30 beetles in each tub. The beetles were fed bananas two days prior to the start of each experiment. Beetles were kept in an air conditioned room when not being used in flight tests.

2.2 Field Cages

Experiments were performed in two custom-designed large field cages (20' x 20' x 10') erected at the University of Guam's Agricultural Experiment Station in Yigo (Fig. 1).

2.3 Attractants and Targets

We obtained two samples from ISCA: SPLAT-RB and SPLAT-RB plus cypermethrin. To each sample, we added a fluorescent tracer dye solution (Rhodamine WT, company) at 5 ml per kg. Targets were created by smearing SPLAT onto an area, about XX cm2 at the center of a 30 cm diameter plastic screen. The screen was supported by a stand at a height of about about 2 m (Fig. 2 and 3).

2.4 Flight Tests

Flight tests were only run during evenings in which the average wind speed was less than 5 mph, as measured by a weather station only 300' from the field cages, and when the probability of rain during the test period was low. At about 30 minutes prior to sunset, a plastic tub containing 30 beetles was put in each cage and the lid was removed. Beetles cannot crawl out of tubs, but they can fly out. Each cage contained a trap hung at about 6 feet above the ground and One cage contained a trap baited with a new lure and the other contained a trap baited with a depleted lure.

After making a decision to run a flight test, at about 30 minutes prior to sunset, a tub containing 30 beetles was placed in each cage and the lid was removed. Location of the trap and the tub were adjusted so that the trap was directly upwind with respect to the tub.

At about three hours after sunset, beetles were collected, counted and returned to their tubs. Beetles which had been trapped and those found elsewhere with the cage were tallied.

3 Results

Figure 1: Large, custum-designed field cages (20' x 20' x 10') used for semifield experiments with the coonut rhinoceros beetle.

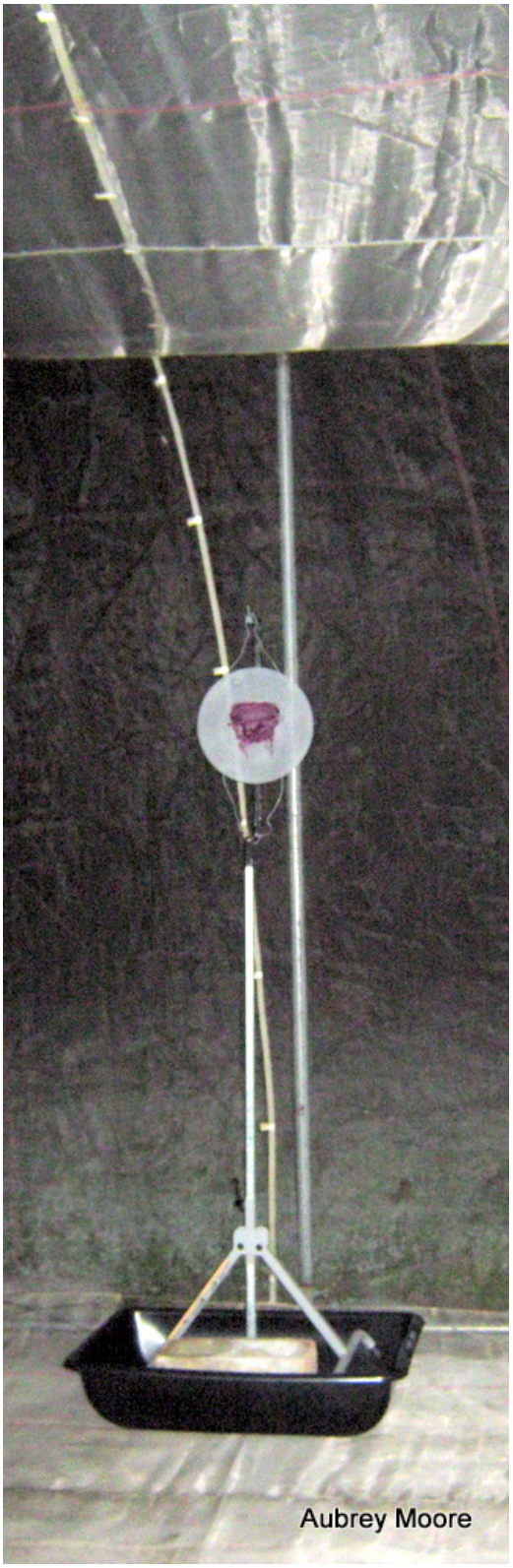


Figure 2: Caption.

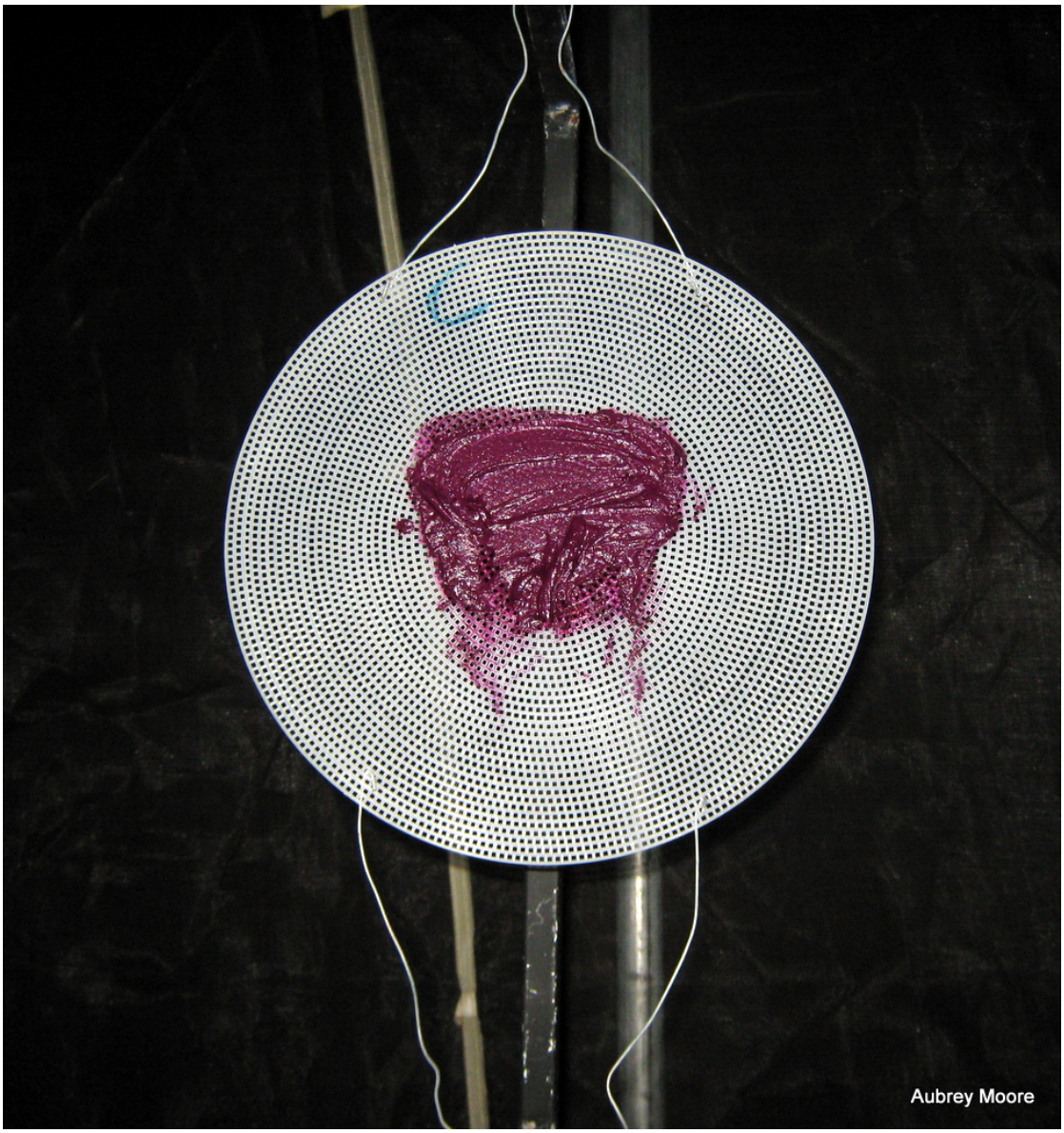


Figure 3: Caption.

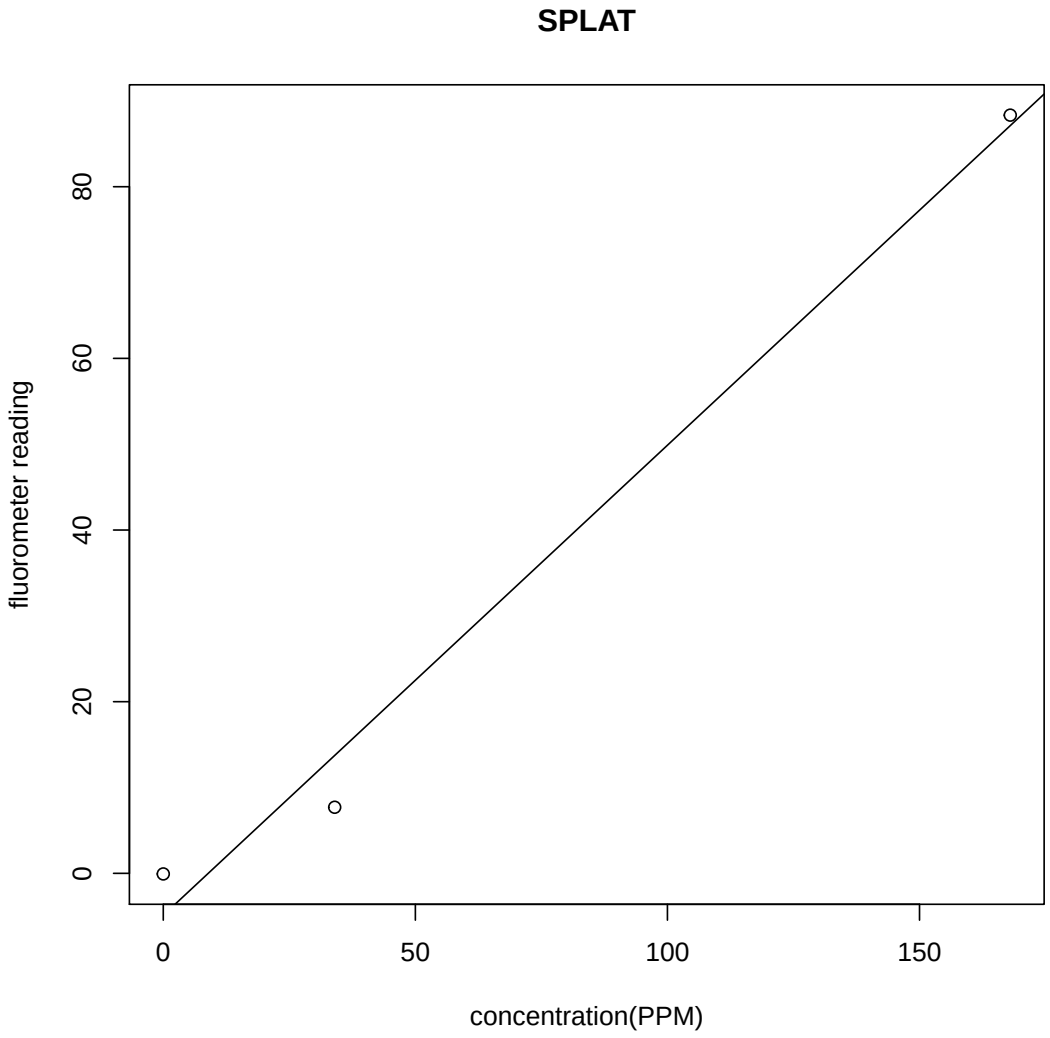


Figure 4: Fluorometer calibration for SPLAT containing rhodamine WT tracer dye. y = -4.8845 + 0.5476x.

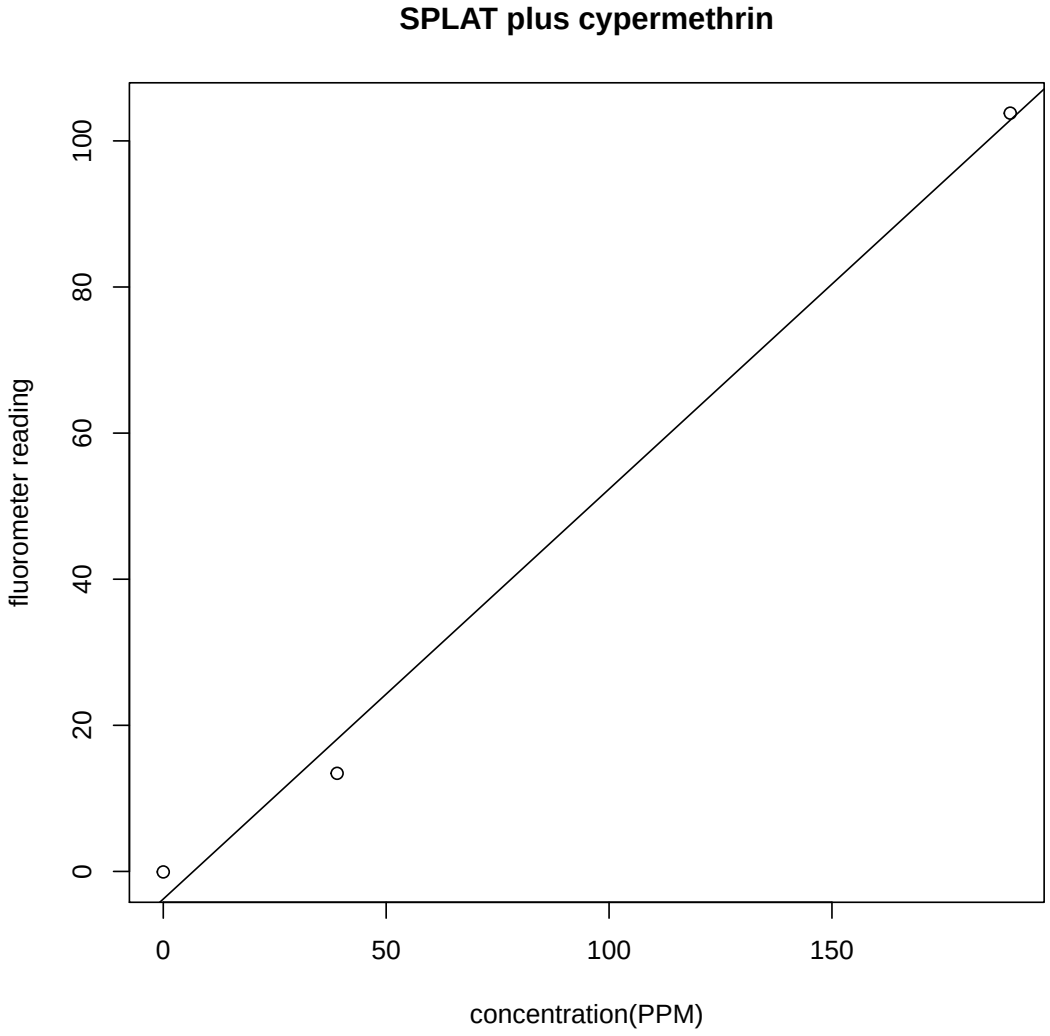


Figure 5: Fluorometer calibration for SPLAT plus cypermethrin containing rhodamine WT tracer dye. y = -3.7835 + 0.5612x.

```
d = sqldf("\nSELECT BeetleID, Fluorometer, Status12h FROM data\nWHERE Expt=1 AND Target=
p = ggplot(d, aes(x = 0.05 * ppm.splat(Fluorometer), y = BeetleID, colour = Status12h)) +
    geom_point(size = 4) + geom_vline(aes(xintercept = 0.05 * ppm.splat(background))) +
    xlab("mg") + xlim(c(0, 12))
p

## Warning:  row names were found from a short variable and have been
## discarded
```

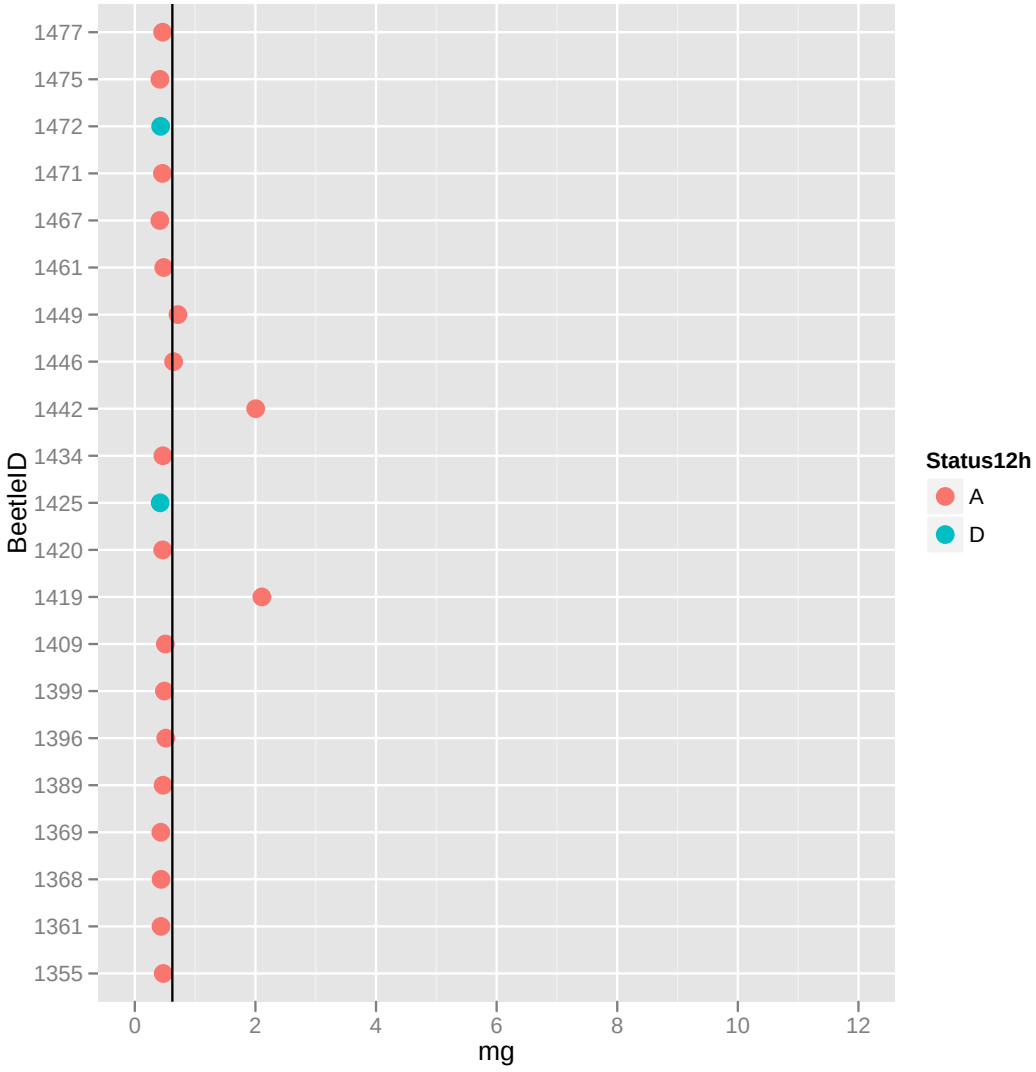


Figure 6: plot-exp1-splat

```
d = sqldf("\nSELECT BeetleID, Fluorometer, Status12h FROM data\nWHERE Expt=1 AND Target="
p = ggplot(d, aes(x = 0.05 * ppm.splatc(Fluorometer), y = BeetleID, colour = Status12h))
    geom_point(size = 4) + geom_vline(aes(xintercept = 0.05 * ppm.splatc(background))) +
    xlab("mg") + xlim(c(0, 12))
p

## Warning:  row names were found from a short variable and have been
## discarded
```

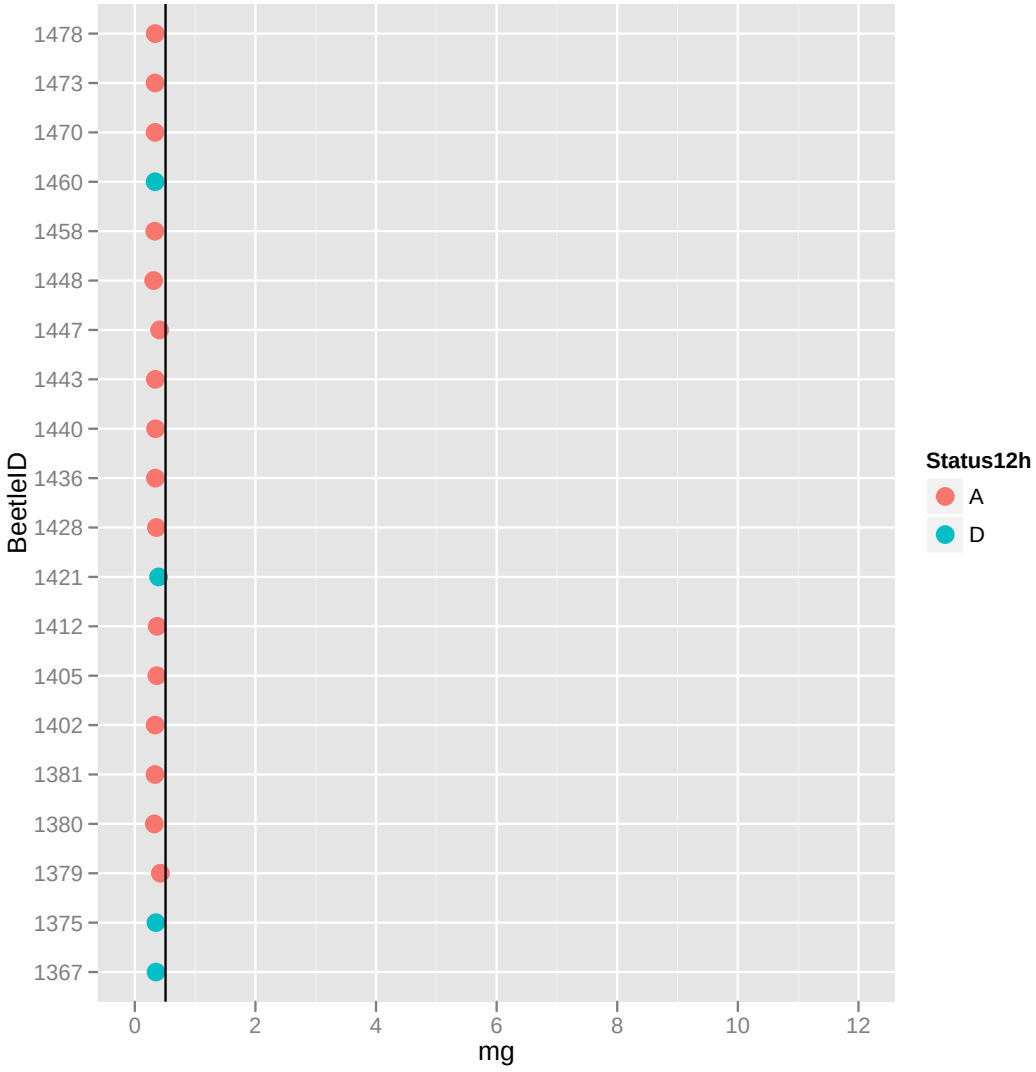


Figure 7: plot-exp1-splatc

```
d = sqldf("\nSELECT BeetleID, Fluorometer, Status12h FROM data\nWHERE Expt=2 AND Target=
p = ggplot(d, aes(x = 0.05 * ppm.splat(Fluorometer), y = BeetleID, colour = Status12h)) +
    geom_point(size = 4) + geom_vline(aes(xintercept = 0.05 * ppm.splat(background))) +
    xlab("mg") + xlim(c(0, 12))
p

## Warning:  row names were found from a short variable and have been
## discarded
```

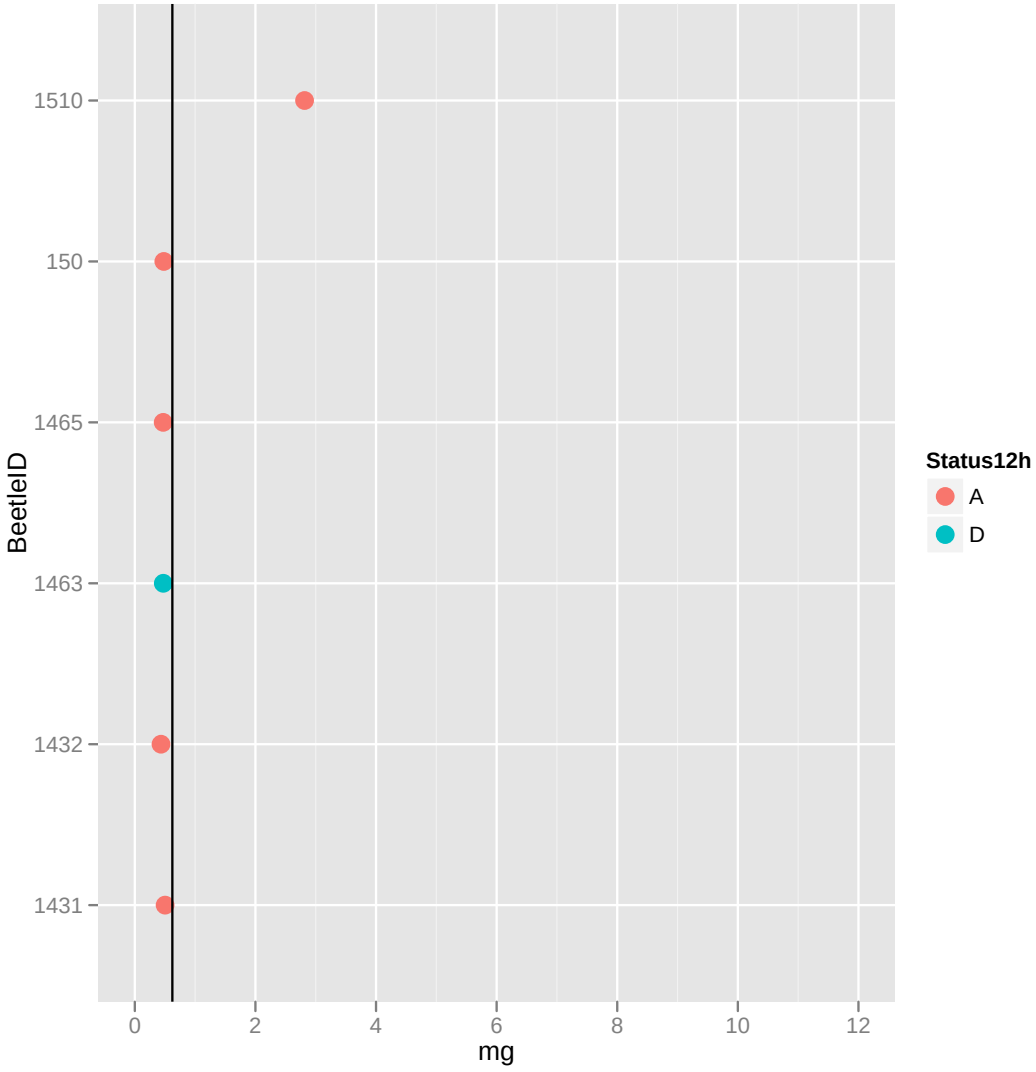


Figure 8: plot-exp2-splat

```
d = sqldf("\nSELECT BeetleID, Fluorometer, Status12h FROM data\nWHERE Expt=2 AND Target="
p = ggplot(d, aes(x = 0.05 * ppm.splatc(Fluorometer), y = BeetleID, colour = Status12h))
    geom_point(size = 4) + geom_vline(aes(xintercept = 0.05 * ppm.splatc(background))) +
    xlab("mg") + xlim(c(0, 12))
p

## Warning:  row names were found from a short variable and have been
## discarded
```

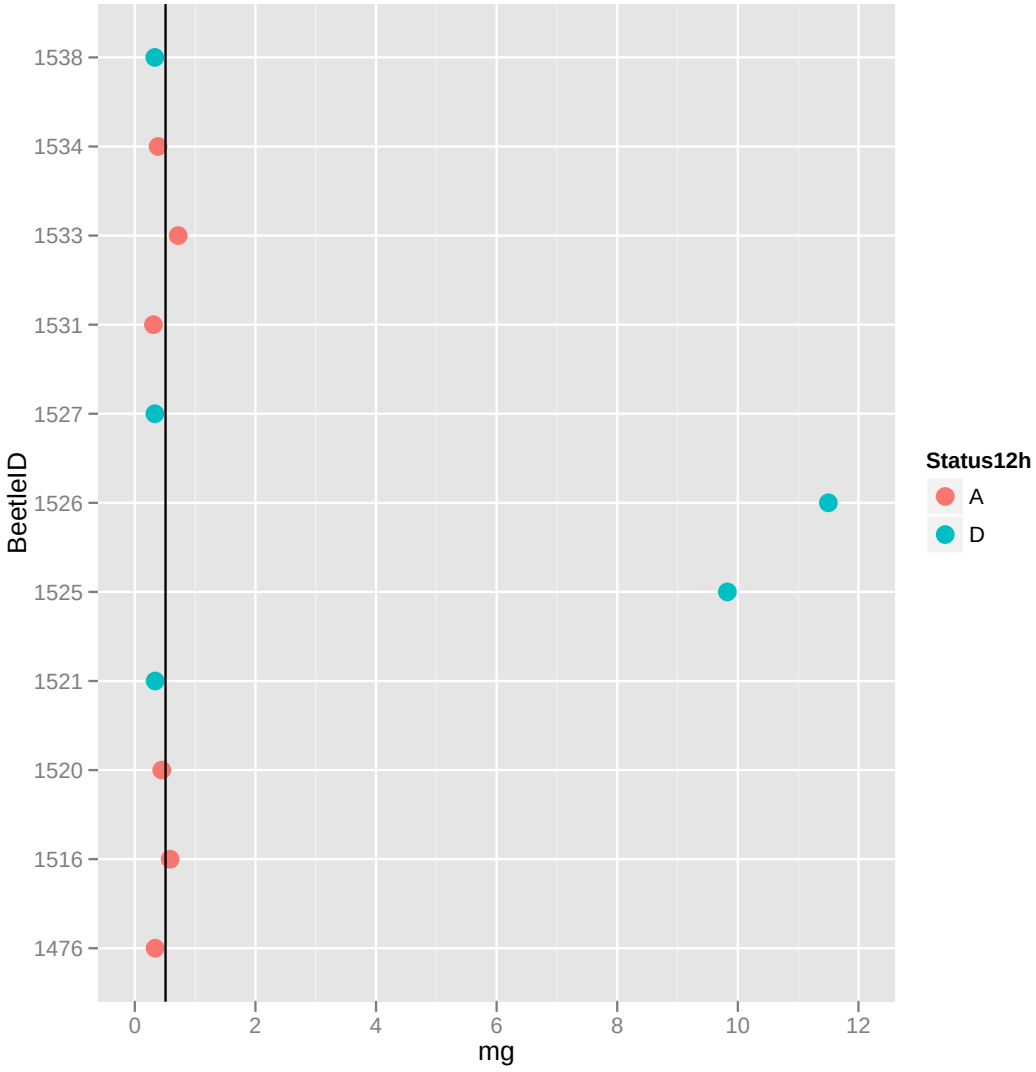
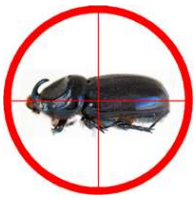


Figure 9: plot-exp2-splatc

8 Appendix: Field Cage Experiment: Escape Test

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Field Cage Experiment
Escape Test

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

October 9, 2012

None of 56 beetles were able to escape from vaned bucket traps.

1 Introduction

The Guam Coconut Rhinoceros Beetle Eradication project uses standard vaned bicket traps. Observations of beetles taking off vertially, 'helicoptering', led us to perform this experiment to confirm that captured beetles are not escaping.

2 Methods

2.1 Beetles

For each experiment, we field collected adult coconut rhinoceros beetles, *Oryctes rhinoceros*. These were housed in two plastic tubs half filled with peat moss, 30 beetles in each tub. The beetles were fed bananas two days prior to the start of each experiment. Beetles were kept in an air conditioned room when not being used in flight tests.

C:/Documents and Settings/Administrator/My Documents/CRB Field Cage/splat/escapeTest.Rnw

2.2 Field Cages

Experiments were performed in two custom-designed large field cages (20' x 20' x 10') erected at the University of Guam's Agricultural Experiment Station in Yigo (Fig. 1).

2.3 Flight Tests

On October 5, 2012 at about 30 minutes prior to sunset, about 26 beetles were placed in new vaned bucket trap which were hung on a stand in cages N and 30 beetes were placed in a bucket trap hung in cage S.

Beetles became active and strted buzzing their wings at about 15 minutes after sunset. At about three hours after sunset, beetles were collected, counted and returned to their tubs.

3 Results

All of 26 beetles placed in the bucket in cage N remained in the bucket. Similarly, all of 30 beetles placed in the bucket in cage S remained in the bucket.

Figure 1: Large, custum-designed field cages (20' x 20' x 10') used for semifield experiments with the coonut rhinoceros beetle.

9 Appendix: Extension Flyer: CRB Control Tips

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

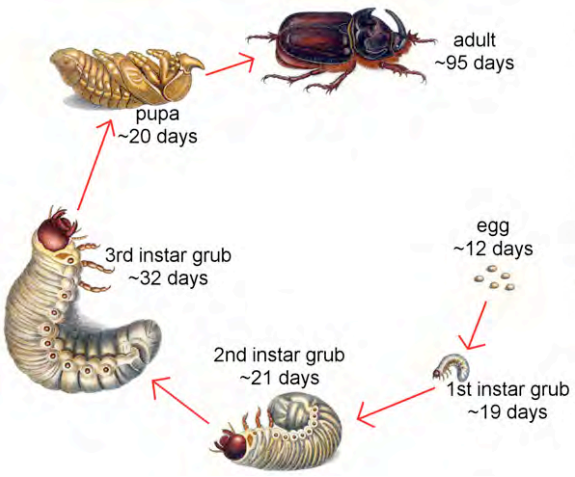
rev. July 10, 2013

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



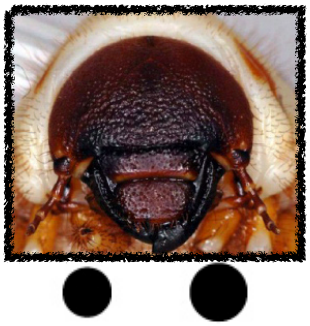
The coconut rhinoceros beetle (CRB), *Oryctes rhinoceros*, is a large scarab beetle that feeds on coconut and other palms. The adult beetles bore holes into the crowns of coconut trees and feed on the sap. This is what causes the distinctive v-shaped cuts in the leaves.

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

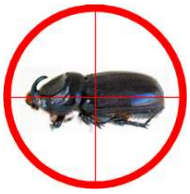


3rd instar
black dots represent the size range of head capsule 9.5 - 11.2 mm

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

10 Appendix: Cypermethrin Applied to Coconut Palm Crowns as a Prophylactic Treatment 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

Noveber 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 November 6, 2013
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.

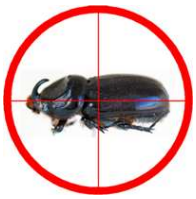
	date	tree
1	2013-05-19	3418
2	2013-05-19	3427
3	2013-05-19	3428
4	2013-05-19	3431
5	2013-05-19	3417
6	2013-05-21	3433
7	2013-05-21	3418
8	2013-05-22	3412
9	2013-05-23	3407
10	2013-05-26	3407
11	2013-05-28	3427
12	2013-06-04	3407
13	2013-06-04	3413
14	2013-06-08	3430
15	2013-06-14	3407
16	2013-06-17	3406
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

11 Appendix: Reduced Release Rate Lures

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



Reduced Release Rate Lures

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

March 29, 2013*

1 Introduction

Two independent pieces of evidence indicate that the release rate for Oryctalure®is two high for use in the baffled bucket traps used for monitoring and potentially controlling the population of CRB adults on Guam:

- In large field cage tests, bucket traps baited with depleted lures (no liquid visible behind membrane) caught as many beetles as traps baited with new lures. The measured release rate for new and depleted lures used in the field cage experiments was 17.02 and 0.39 mg/day, respectively. In other words, depleted lures emit only about 2% as much pheromone as the new lures.
- Long-term trap catch records from the Guam trapping network (about 1000 traps) indicate that depleted lures catch about 2.5 times the number of beetles caught in traps with new lures

A field experiment is being planned to test trap modifications. One factor to be tested is a reduced release rate lure. For this experiment, we want to attenuate the Oryctalure®release rate to about 5% of the current rate.

*Revised April 29, 2013
C:/Documents and Settings/Administrator/My Documents/CRB Tech Reports/ReducedReleaseRate

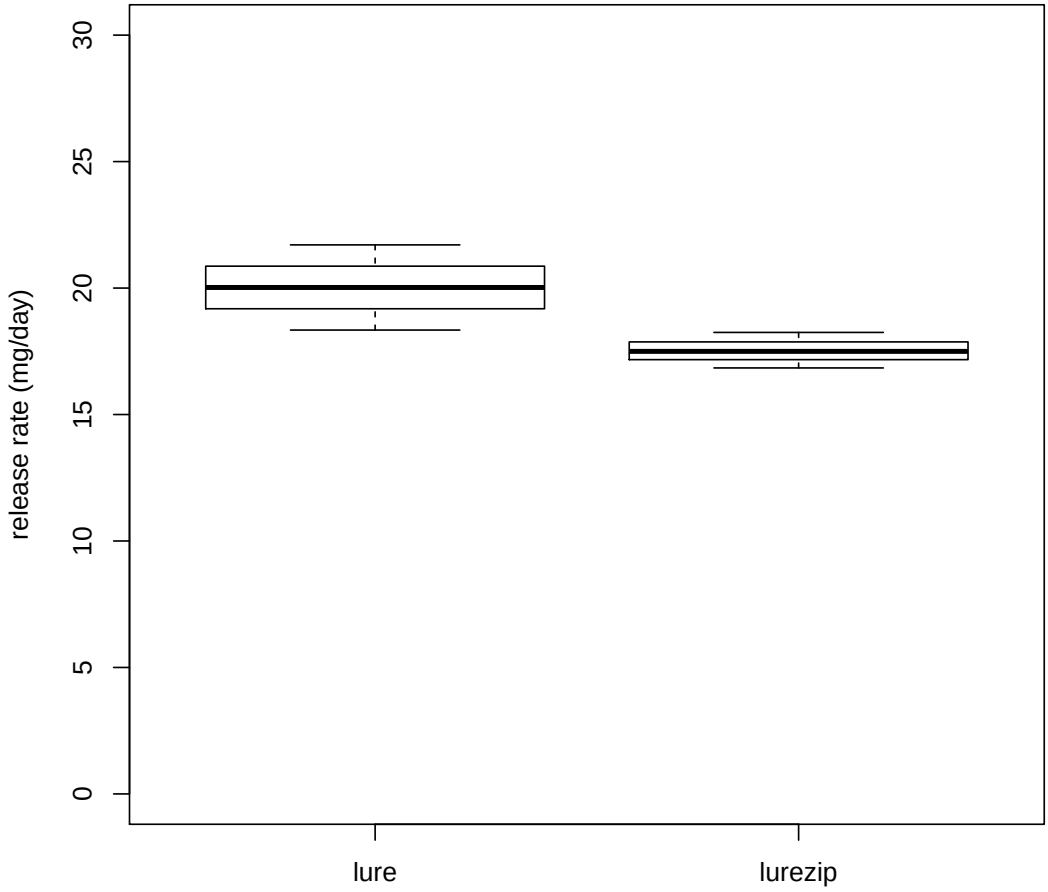
2 Methods

Attempted to reduce the release rate by placing lures in ziploc plastic bags (Fig. 1). Three new lures and three new lures sealed in ziploc bags were hung outdoors in a shady area for one week. Gross eight of each lure was measured before and after the exposure period.

Figure 1: Attempt at reducing release rate by putting lure in a ziploc bag.

3 Results

```
t1 = strptime("2013-03-21 16:20", "%Y-%m-%d %H:%M")
t2 = strptime("2013-03-28 15:10", "%Y-%m-%d %H:%M")
t3 = strptime("2013-04-01 08:50", "%Y-%m-%d %H:%M")
days = as.numeric(difftime(t3, t1))
treatment = c(rep("lure", 3), rep("lurezip", 3))
id = c("1", "2", "3", "1b", "2b", "3b")
m1 = c(3107, 3055, 3069, 3608, 4019, 3849)
m2 = c(2928, 2901, 2906, 3455, 3867, 3709)
m3 = c(2875, 2859, 2855, 3413, 3832, 3669)
mg.per.day = (m1 - m3)/days
mg.per.day

## [1] 21.71 18.34 20.02 18.25 17.50 16.84

tt = t.test(mg.per.day ~ treatment)
tt

##
##  Welch Two Sample t-test
##
## data:  mg.per.day by treatment
## t = 2.368, df = 2.675, p-value = 0.1092
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -1.100  6.091
## sample estimates:
##    mean in group lure mean in group lurezip
##                 20.02                 17.53
##

boxplot(mg.per.day ~ treatment, ylim = c(0, 30), ylab = "release rate (mg/day)")
```



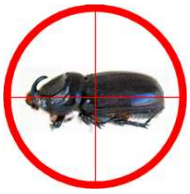

```
# paste('Release rate of lures in ziplog bags is ', round(100*21.34/23.78,
# 1), 'percent of standard lures.')
pt1 = strptime("2013-03-30 06:40", "%Y-%m-%d %H:%M")
pt2 = t3
pt.rr = (21788 - 21786)/as.numeric(difftime(pt2, pt1))
pt.rr

## [1] 0.9568
```

4 Discussion

The plastic ziploc bags reduced the release rate of the lures by only about 10%. In fact, the measured difference in release rates was not significantly different from zero. The goal is about 95% attenuation.

12 Appendix: 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
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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.

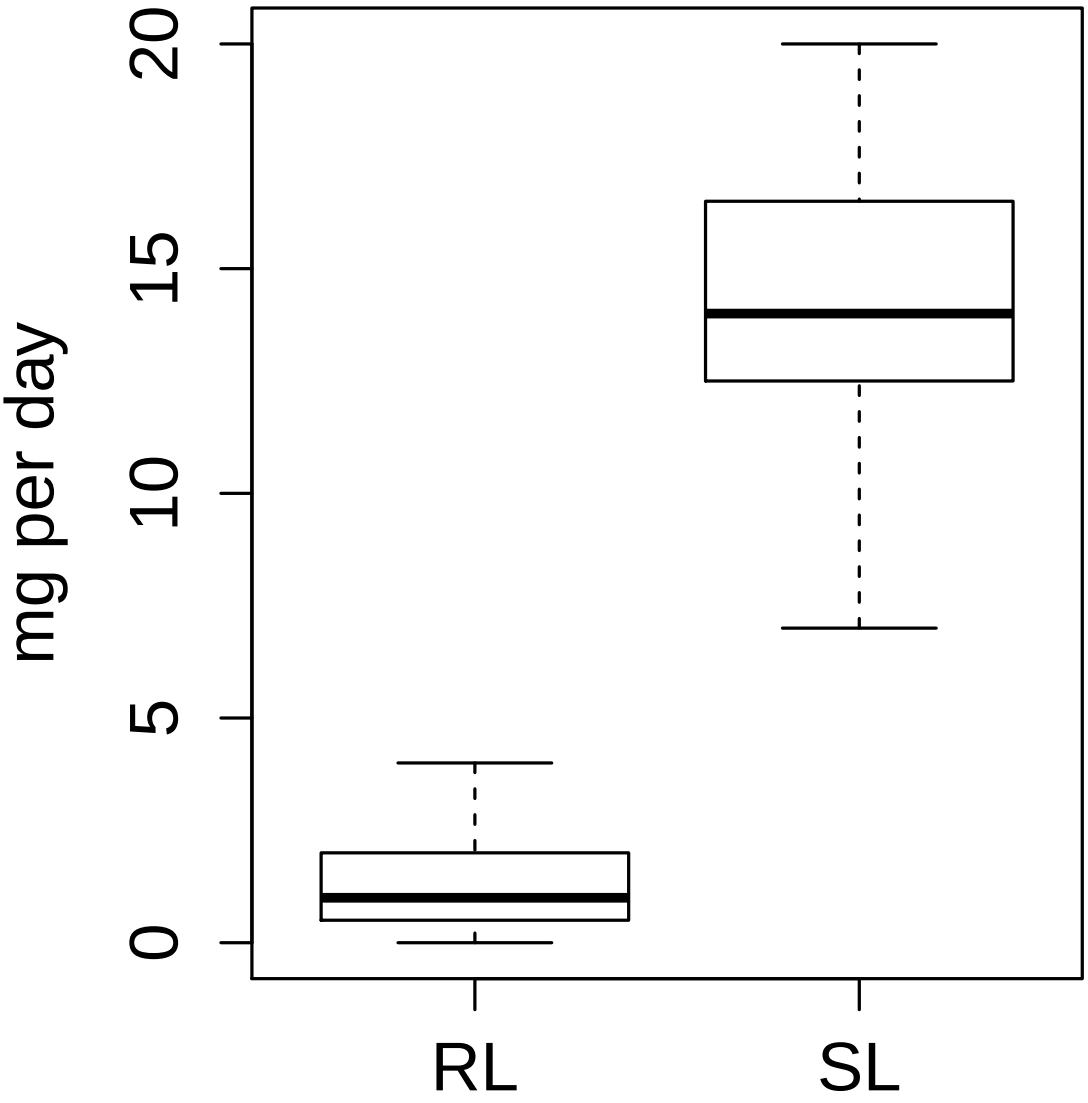


Figure 2: Release rates for standard and reduced rate lures.

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.

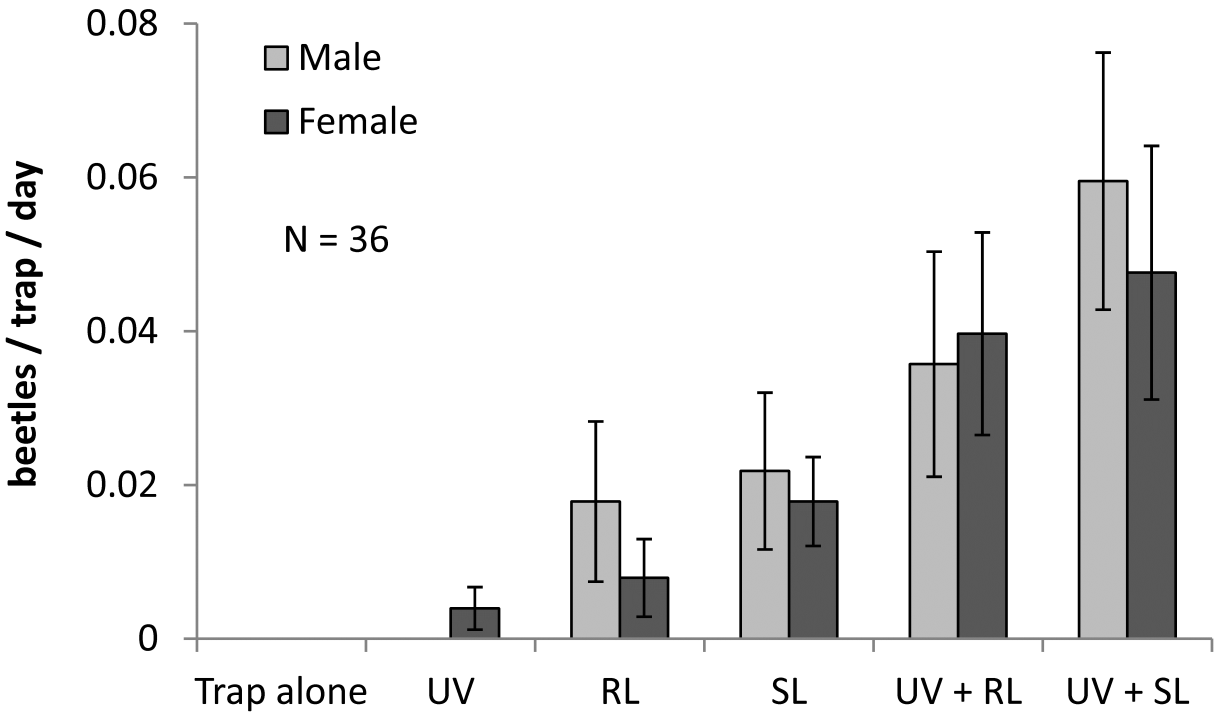
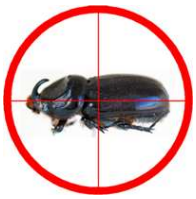


Figure 3: Mean daily trap catch for each trap type.

13 Appendix: One Way Mesh

Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project



One-way Top for Barrels

Prepared by
Aubrey Moore
University of Guam Cooperative Extension Service

April 29, 2013

1 Introduction

Oil barrels filled with decaying coconut material are being used as artificial breeding sites ('sinks'). This experiment tests the idea of covering the top of th barrel with chicken wire which will let beetles enter but not leave.

2 Methods

On April 25, 2013, two empty oil barrels were placed in one of the large (20' x 20' x 10') field cages (Fig. 1). The top of one barrel was covered with hexagonal pattern chicken wire screen with about a one inch mesh size (Fig. 2). The other barrel was left open.

At dusk, pan containing adult rhino beetles in damp peat moss was opened and place on the ground at the center of the cage. As beetles flew out, they were captured and alternately placed in the screened barrel and the unscreened barrel. During the evening, 6 beetles were placed in each barrel.

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Figure 1: Experimental setup.

Figure 2: Rhino beetle on chicken wire mesh.

3 Results

By the end of the flight period, about 10:00 pm, 5 of 6 beetles had escaped from the unscreened barrel and 0 of 6 had escaped from the screened barrel. Two days later, the remaining beetle had escaped from the unscreened barrel and 0 of 6 beetles had escaped from the screened cage.

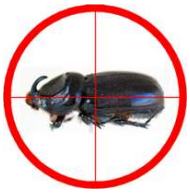
Direct observation showed that beetles easily enter the barrel through the screen but where deflected back into the barrel when they tried to fly out because the wing span of these beetles is much larger than the mesh size of the chicken wire (Fig. 2).

4 Discussion

Chicken wire tops for artificial breeding sites allows adult rhino beetles to enter, but not leave. Securing the chicken wire to the top of the barrel is tedious. Perhaps 'barrel hoops' can be faricated to make the job easier.

14 Appendix: 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 November 6, 2013
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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 caught 54% more beetles.

Figure 1: CRB barrel trap.

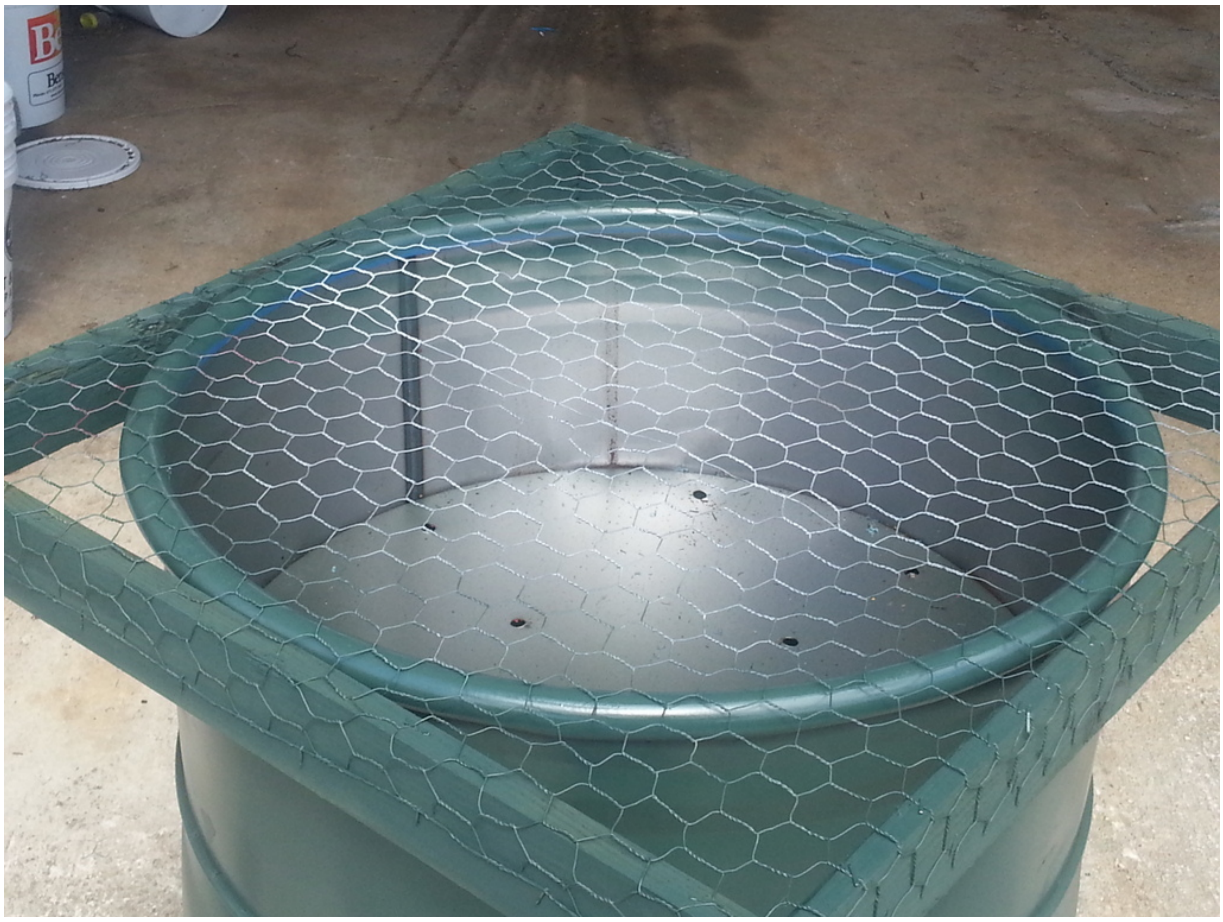

Figure 2: CRB barrel trap fitted with a pan to facilitate counting newly arrived adults.