Progress on Objectives as Stated in the Work Plan for the USDA-Forestry Service Grant Entitled "Support for the Guam Coconut Rhinoceros Beetle Eradication Project" (Agreement 11-DG-11052012-101)

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Contents

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: 70%

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.

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: 50%

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.

To do:

- Use trail cams 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, four 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
- A downward trend in average rhino beetle trap catch coincided with the start of widespread dispersal of *Metarhizium* in November 2011. 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: 10%

Progress:

- This objective will recieve 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 are being set up to test these on Guam

To Do:

- Perform lab bioassays on new virus samples from New Zealand and Fiji

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: 0%

Progress:

- This simple experiment is on hold until other experiments which require use of the large field cages are completed.
- 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.

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.

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: 75%

Progress:

- A team of insect chemical ecologists under the leadership of Eric Jang, USDA-ARS Pacific Basin Research Center visited Guam during May 2012. 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.

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:

- During the performance period for the current project, 947 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 on May 23, 2011, 12,831 trap visits were made and 3640 adult beetles were trapped. The infestation has spread to most parts of the island. However, average trap catch is relatively low (<x beetles per trap-day) and the upward trend ceased in November, 2011 (Figure 1). This coincided with release of *Metarhizium majus* biocontrol agent into the population. It is possible that the *Metarhizium* is providing density-dependent control, thus limiting the population explosion.
- The project's sanitation crew found and destroy 1052 adult beetles and 8,647 immatures. Thirty-eight infested trees were felled and destroyed.
- 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 100 rhino beetle breeding sites.

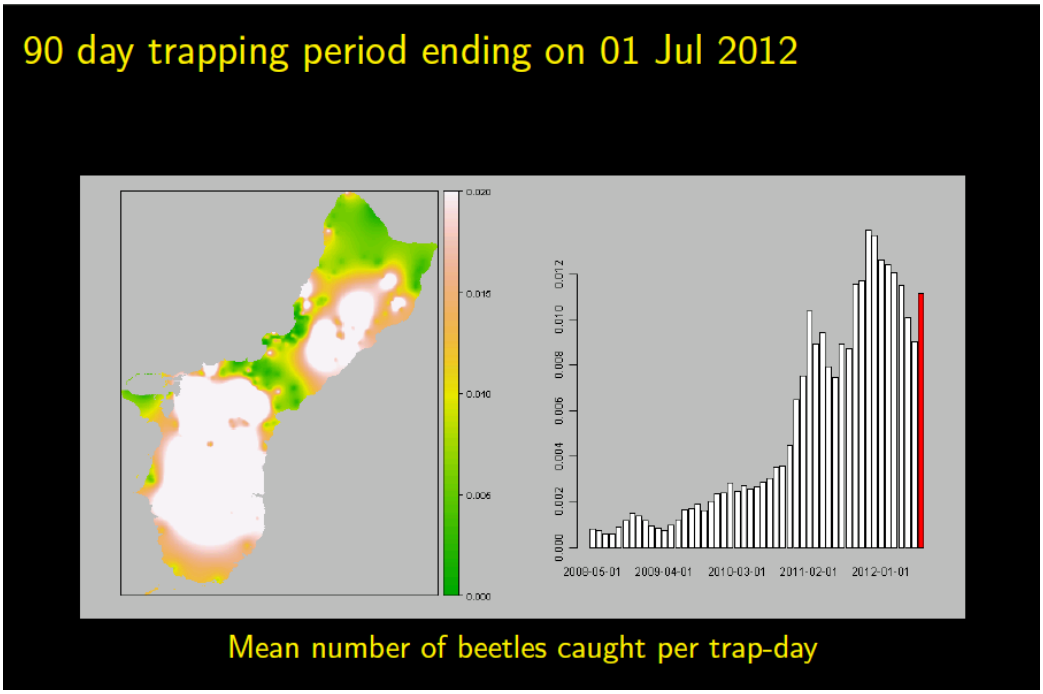

Figure 1: Spatial-temporal display of coconut rhinoceros trap data.

- The projects rearing facility is operating well and is keeping up with demands for experimental animals. Freshly trapped male adult beetles are currently being used for autodissemination of *Metarhizium* instead of reared individuals.

5 Travel

In September, 2011, Aubrey Moore traveled to Legaspi, Philippines to visit with Dr. Ambrose Alfiler at the Philippine Coconut Authority Albay Research Center. Arrangements were made for Dr. Legaspi to provide *Metarhizium majus* spores for biocontrol of the coconut rhinoceros beetle on Guam under conditions of an approved USDA-APHIS import and release permit.

In November, 2011, Aubrey Moore and Roland Quitugua participated in the Society of American Foresters Annual Convention in Honolulu, Hawaii. They presented an oral presentation on the Guam Coconut Rhinoceros Beetle

Eradication Project and participated in a session on invasive species impacting forestry on Pacific islands.