

PPA19 Report 1: Coconut Rhinoceros Beetle Biological Control

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1. Staffing

Staff for this project currently comprises of the PI, a post-doc and a technician.

- Funding from the Department of Interior, Office of Island Affairs was used to hire an insect pathologist for a 2 year term. Dr. James Grasela was recruited and started work at UOG on June 24, 2018.
- Mr. Chris Cayanan was hired as a technician for this project on DATE.

2. Laboratory Bioassays of OrNV

The major goal of this project is to find an effective biological control agent for coconut rhinoceros beetle biotype G (CRB-G).

Prior to arrival of CRB on Guam during 2007, coconut rhinoceros beetle infestations of Pacific islands were readily controlled by classical biological control using *Oryctes nudivirus* (OrNV). Following a lack of response to release of OrNV on Guam, research showed that the Guam CRB population is a genetically distinct virus-resistant biotype which has become known as CRB-G. This biotype is highly invasive and is causing massive damage to coconut and oil palms in Papua New Guinea and the Solomon Islands. CRB-G has also invaded Oahu and Rota. Eradication attempts have been launched on these two islands.

The current project is part of an informal international collaboration among Pacific island entomologist working to find solutions for CRB-G management. The current focus is on testing new OrNV isolates in the hope of finding one or more that can be used as an effective biological control agent for CRB-G.

2.1. Haemocoel Injection Bioassays

In this series of assays, we tested 4 isolates of OrNV which had produced by an insect cell culture at the AgResearch Laboratory in New Zealand. Adult beetles were dosed by direct injection into the haemocoel. This series is a preliminary test for pathogenicity. Insignificant differences in mortality curves between virus treatment and the other two treatments (control treatment and heat-inactivated virus) is an indicator of pathogenicity. Gut tissue samples have been preserved for histology and molecular analysis.

The following is a brief summary of results. Details are provided in the appended technical reports. Results indicate that isolates DUG42 and MALB are not pathogenic for CRB-G, but isolates PNG and V23B are pathogenic. Bioassays in which adult beetles are dosed *per os* are underway and results will be available in a future report.

2.1.1. OrNV Isolate DUG42

Origin: Philippines; 2 replicates; 30 beetles in total

No significant differences among mortality curves. [control, heat-inactivated virus, virus]

2.1.2. OrNV Isolate MALB

Origin: Malaysia; 2 replicates; 30 beetles in total

No significant differences among mortality curves. [control, heat-inactivated virus, virus]

2.1.3. OrNV Isolate PNG

Origin: Papua New Guinea; 4 replicates; 71 beetles in total

Mortality curves in 2 significantly different groups: [control, heat-inactivated virus], [virus]

2.1.4. OrNV Isolate V23B

Origin: Solomon Islands; 4 replicates; 66 beetles in total

Mortality curves in 2 significantly different groups: [control, heat-inactivated virus], [virus]

2.2. PCR

Grasela and Moore [2020a](#) Grasela and Moore [2020b](#) Grasela and Moore [2020c](#)

3. Environmental Cabinets and CRB Rearing

Three environmental cabinets which allow control of temperature, relative humidity, and lighting for insect rearing were procured and installed. These chambers are set to maintain 30°C, 80% RH and 12h photoperiod.

After a power outage caused by a typhoon, one of the cabinets malfunctioned. It heated beyond the setpoint and killed all beetles. To prevent this problem from recurring, controllers for all three units have been programmed to send email to project staff whenever a fault is detected.

The project does not currently rear beetles from egg to adult. Because CRB are so numerous on Guam, it is far more efficient to field collect prepupae, pupae and adults and rear these to the age required for bioassays. Adults are fed banana slices.

4. CRB Damage Survey

- A 360 degree digital camera and accessories were purchased.
- A protocol for roadside CRB damage surveys using digital imagery was developed and trial runs were made.
- A workflow for scoring CRB damage from digital imagery is under development.

5. Regional Collaboration

An informal collaboration, the *CRB-G Action Group*, has been formed among Pacific-based entomologists working on the CRB-G problem. Participants from Guam, Hawaii, Palau, Papua New Guinea, Solomon Islands, Fiji, Malaysia, Japan and New Zealand have met several times and future meetings are planned (Table 1). This is an *ad hoc* group which has been organized by Dr. Trevor Jackson and Sean Marshall of AgResearch New Zealand. AgResearch is recognized as a global center for expertise on biological control of CRB. AgResearch scientists have worked on CRB in the south Pacific for several decades and they maintain a library of OrNV isolates in cell culture. The New Zealand government has recently committed several million dollars to aid response to CRB-G in the south Pacific islands.

Although individual institutions working to find a solution to the CRB-G problem on American-affiliated islands in the northern Pacific have secured funding from multiple, short-term grants, attempts to secure funding to support a sustainable well-coordinated regional project have been unsuccessful. We respectfully request SERDP funding to support collaboration and cooperation on progress towards solving the CRB-G problem among partners in the American Pacific in partnership with international colleagues in the south Pacific. If our request is granted the project PI and staff at NCSU would be tasked with organizing quarterly Pacific-wide teleconferences and helping to organize and fund participation from the American Pacific in annual CRB-G Action Group conferences in 2021 through 2024.

Table 1: Meetings of the CRB-G Action Group

2015 Pacific Entomology Conference, Honolulu, HI, USA
2016 International Congress of Entomology, Orlando, USA
2017 Japanese Society for Insect Pathology, Tokyo, Japan
2018 Society for Invertebrate Pathology, Gold Coast, Australia
2019 XIX International Plant Protection Congress, Hyderabad, India
2020 (tentative): Pacific Plant Protection Organization, Guam

5.1. Participation in Scientific Meetings

Moore and Grasela participated at the MEETING in a symposium entitled The challenge of coconut rhinoceros beetle , *Oryctes rhinoceros*, to palm production and prospects for control in a changing world.

They also participated in a Coconut Rhinoceros Beetle Action Group meeting.

Presentation Moore, Grasela, and Sean D. G. Marshall [2019](#)

Presentation Sean David Goldie Marshall [2019](#)

5.2. Development of Online Resources

6. Appendices

A. Appendix A: Technical Report: Injection Bioassay of OrNV Isolate DUG42

See following page.

B. Appendix B: Technical Report: Injection Bioassay of OrNV Isolate MALB

See following page.

C. Appendix C: Technical Report: Injection Bioassay of OrNV Isolate PNG

See following page.

D. Appendix D: Technical Report: Injection Bioassay of OrNV Isolate V23B

See following page.

References

- Grasela, James J. and Aubrey Moore (2020a). *Technical Report: Polymerase Chain Reaction (PCR) Analysis of the Coconut Rhinoceros Beetle (CRB), Oryctes Rhinoceros. Part 1.* URL: <https://github.com/aubreymoore/FY19-PPA-Report-1/blob/master/pcrdata/PCR-Summary-Part-1.pdf>.
- Grasela, James J. and Aubrey Moore (2020b). *Technical Report: Polymerase Chain Reaction (PCR) Analysis of the Coconut Rhinoceros Beetle (CRB), Oryctes Rhinoceros. Part 2.* URL: <https://github.com/aubreymoore/FY19-PPA-Report-1/blob/master/pcrdata/PCR-Summary-Part-2.pdf>.
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- Moore, Aubrey, James J. Grasela, and Sean D. G. Marshall (2019). *Presentation: Status of a Major Outbreak of Coconut Rhinoceros Beetle, Oryctes Rhinoceros Biotype G, on Guam and Attempts at Establishing Biological Control. XIX International Plant Protection Congress, Hyderabad, India. Nov. 11, 2019.* URL: https://github.com/aubreymoore/IAPPS-2019-Presentation/raw/master/Moore_IAPPS-2019.odp.