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Progress Report 3
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Coconut Rhinoceros Beetle Biological Control

Aubrey Moore, University of Guam

March 25, 2019

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1 Staffing

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

- 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.
- Ian Iriarte, my graduate student and technician returned to the University of Guam to work on this project on April 11, 2018. Ian is developing a coconut rhinoceros beetle damage assessment survey, which is one of the objectives of this project, as his Master's thesis topic.

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 nudivorus (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 entomologists 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 been 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]

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

Dr. Madoka Nakai, an insect pathologist from Tokyo University and Dr. Shin Asano, an insect pathologist from Hokkaido University visited Guam in November 2018 to collect CRB-G specimens.

Dr. Nakai returned to Guam in February 2019 with two students to collect additional CRB-G specimens.

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

See following page.



University of Guam Coconut Rhinoceros Beetle Biological Control Project
Bioassay Report generated by CRB Rearing Database v.20190317
<https://aubreymoore.pythonanywhere.com/rearing>

DUG42

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University of Guam Coconut Rhinoceros Beetle Biocontrol Project

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1 Description

Adult beetles incubated at 30°C and 80% RH for more than 2 weeks to observe possible contamination from green muscardine fungus infection were employed in a bioassay to determine the susceptibility of adults to infection by a virus isolate collected from the Philippines (**Dug42**). Treatment 1 consisted of 10-20 μ l sterile filtered water injected at a point on the ventral surface at the junction of the hind leg and the thoracic using a sterile needle. Treatment 2 consisted of 10-20 μ l heat-inactivated virus injection while in the treatment 3, beetles were injected with 10-20 μ l of untreated virus preparation. Adults were then placed in clean glass mason jars (bleach-treated) with a piece of banana added for food. Beetles were incubated at 30°C and 80% RH in a Percival incubator. All beetles were weighted every other day but monitored daily for four weeks to observe any possible signs of mortality.

1.1 Replicate 1

A total of 10 adult females and five adult males distributed among the three treatments were employed in this replicate.

1.2 Replicate 2

A total of seven adult females and eight adult males distributed among the three treatments were employed in this replicate.

2 Mortality

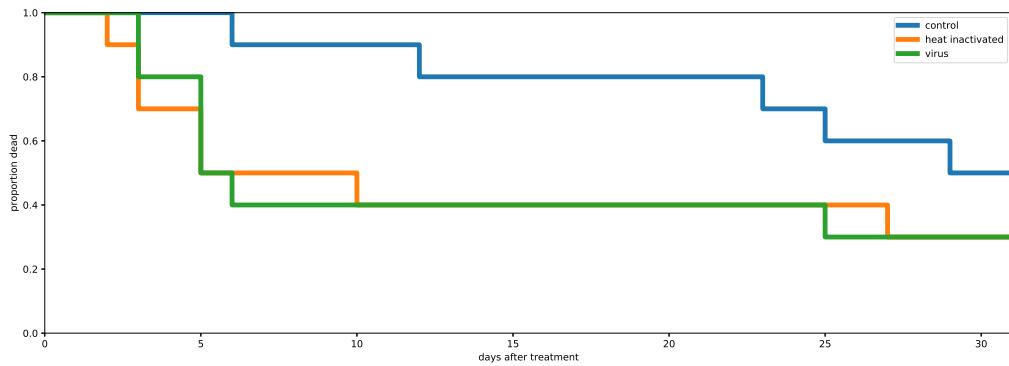
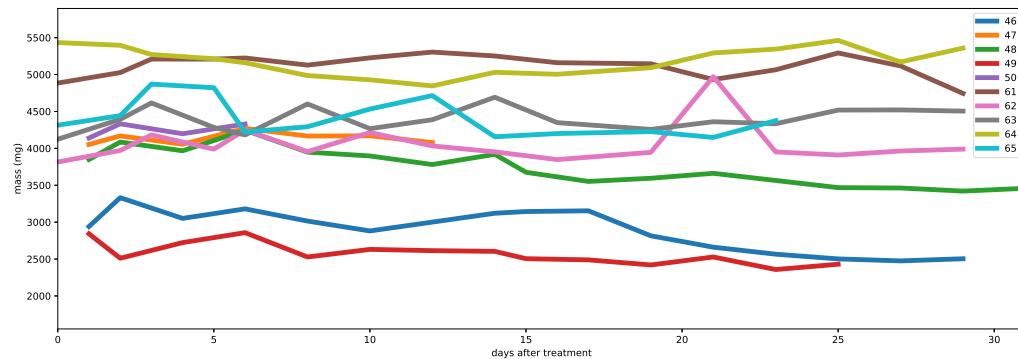


Table 1: Pairwise differences among mortality curves.

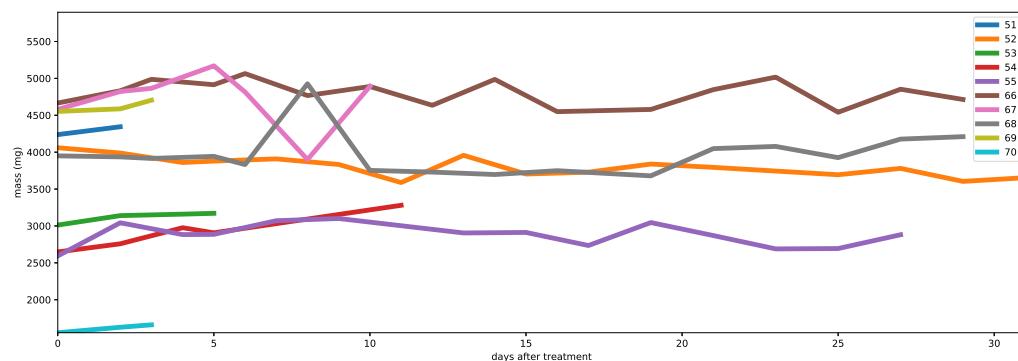
		test_statistic	p
control	heat inactivated	1.920677	0.165782
	virus	2.020872	0.155150
heat inactivated	virus	0.007094	0.932879

3 Mass

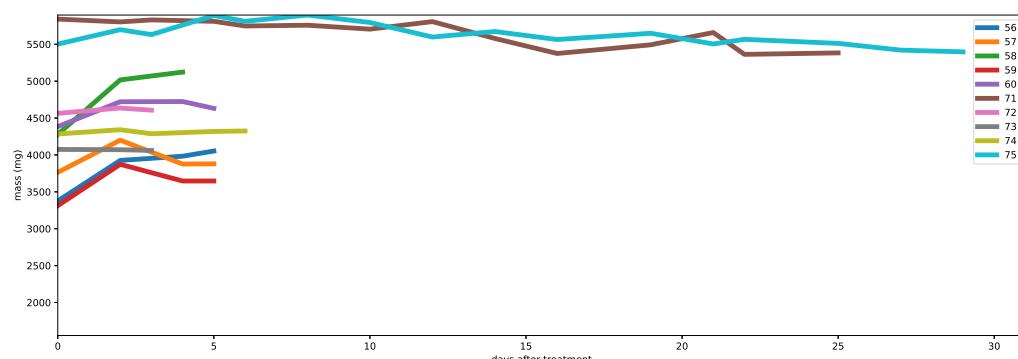
mass control.pdf



mass heat inactivated.pdf



mass virus.pdf



4 Postmortem Images

4.1 control



Figure 1: Bioassay: DUG42-1; Treatment: control; Beetle ID: 46



Figure 2: Bioassay: DUG42-1; Treatment: control; Beetle ID: 47



Figure 3: Bioassay: DUG42-1; Treatment: control; Beetle ID: 48



Figure 4: Bioassay: DUG42-1; Treatment: control; Beetle ID: 49



Figure 5: Bioassay: DUG42-1; Treatment: control; Beetle ID: 50



Figure 6: Bioassay: DUG42-2; Treatment: control; Beetle ID: 61



Figure 7: Bioassay: DUG42-2; Treatment: control; Beetle ID: 62



Figure 8: Bioassay: DUG42-2; Treatment: control; Beetle ID: 63



Figure 9: Bioassay: DUG42-2; Treatment: control; Beetle ID: 64



Figure 10: Bioassay: DUG42-2; Treatment: control; Beetle ID: 65

4.2 heat inactivated



Figure 11: Bioassay: DUG42-1; Treatment: heat inactivated; Beetle ID: 51



Figure 12: Bioassay: DUG42-1; Treatment: heat inactivated; Beetle ID: 52



Figure 13: Bioassay: DUG42-1; Treatment: heat inactivated; Beetle ID: 53



Figure 14: Bioassay: DUG42-1; Treatment: heat inactivated; Beetle ID: 54



Figure 15: Bioassay: DUG42-1; Treatment: heat inactivated; Beetle ID: 55



Figure 16: Bioassay: DUG42-2; Treatment: heat inactivated; Beetle ID: 66



Figure 17: Bioassay: DUG42-2; Treatment: heat inactivated; Beetle ID: 67



Figure 18: Bioassay: DUG42-2; Treatment: heat inactivated; Beetle ID: 68



Figure 19: Bioassay: DUG42-2; Treatment: heat inactivated; Beetle ID: 69



Figure 20: Bioassay: DUG42-2; Treatment: heat inactivated; Beetle ID: 70

4.3 virus



Figure 21: Bioassay: DUG42-1; Treatment: virus; Beetle ID: 56



Figure 22: Bioassay: DUG42-1; Treatment: virus; Beetle ID: 57



Figure 23: Bioassay: DUG42-1; Treatment: virus; Beetle ID: 58



Figure 24: Bioassay: DUG42-1; Treatment: virus; Beetle ID: 59



Figure 25: Bioassay: DUG42-1; Treatment: virus; Beetle ID: 60

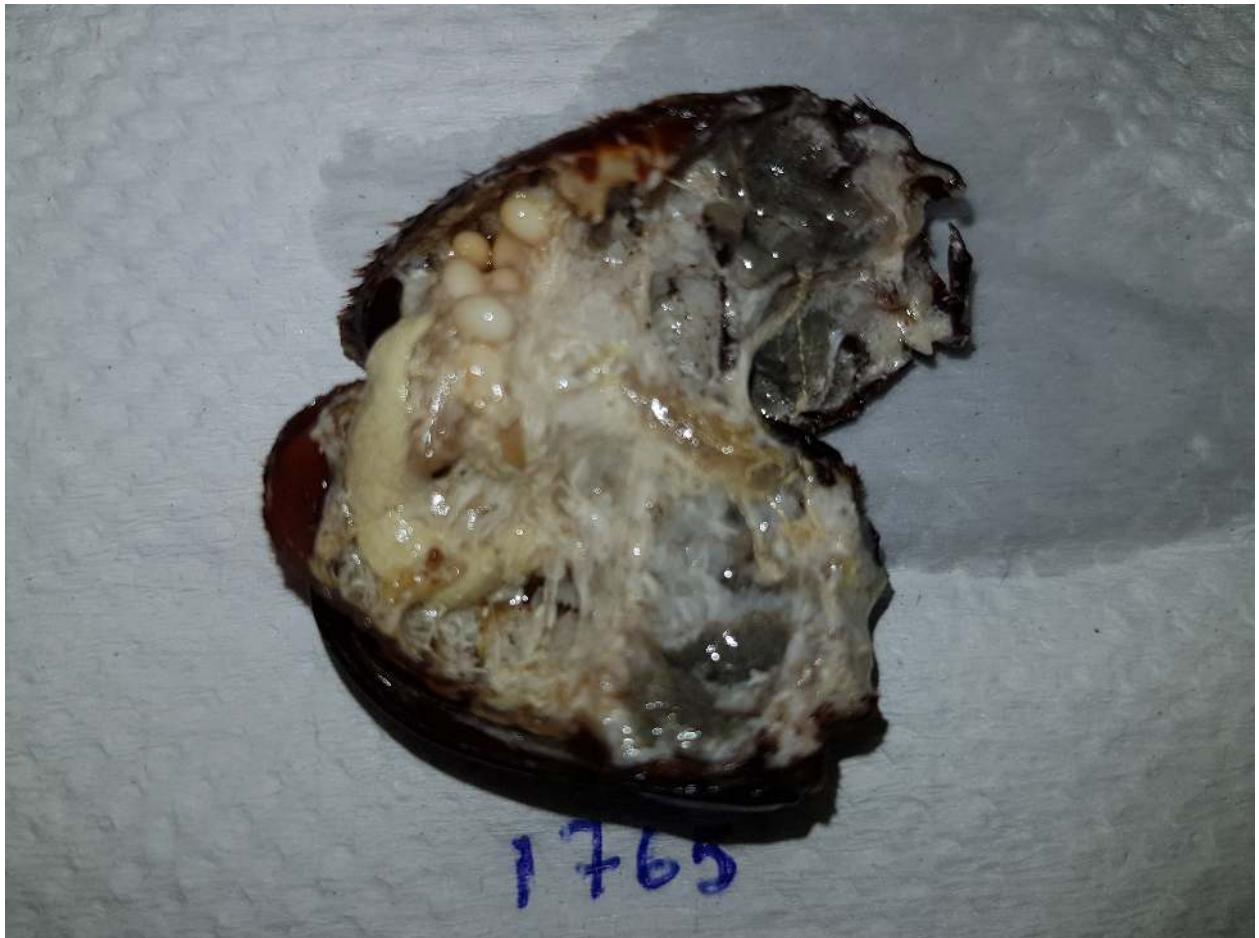


Figure 26: Bioassay: DUG42-2; Treatment: virus; Beetle ID: 71



Figure 27: Bioassay: DUG42-2; Treatment: virus; Beetle ID: 72



Figure 28: Bioassay: DUG42-2; Treatment: virus; Beetle ID: 73



Figure 29: Bioassay: DUG42-2; Treatment: virus; Beetle ID: 74



Figure 30: Bioassay: DUG42-2; Treatment: virus; Beetle ID: 75

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

See following page.



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MALB

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1 Description

Adult beetles incubated at 30°C and 80% RH for more than 2 weeks to observe possible contamination from green muscardine fungus infection were employed in a bioassay to determine the susceptibility of adults to infection by a virus isolate collected from Malaysia (**Mal B**). Treatment 1 consisted of 10-20 µl sterile filtered water injected at a point on the ventral surface at the junction of the hind leg and the thoracic using a sterile needle. Treatment 2 consisted of 10-20 µl heat-inactivated virus injection while in the treatment 3, beetles were injected with 10-20 µl of untreated virus preparation. Adults were then placed in clean glass mason jars (bleach-treated) with a piece of banana added for food. Beetles were incubated at 30°C and 80% RH in a Percival incubator. All beetles were weighted every other day but monitored daily for four weeks to observe any possible signs of mortality.

1.1 Replicate 1

A total of nine adult females and six adult males distributed among the three treatments were employed in this replicate.

1.2 Replicate 2

A total of nine adult females and six adult males distributed among the three treatments were employed in this replicate.

2 Mortality

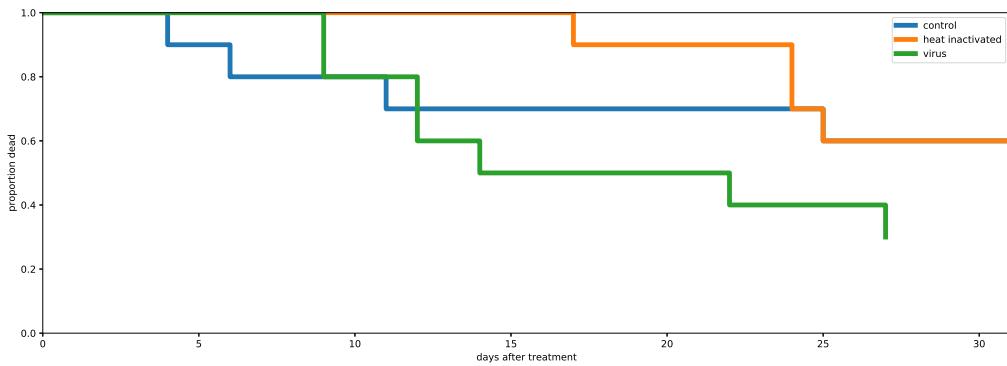
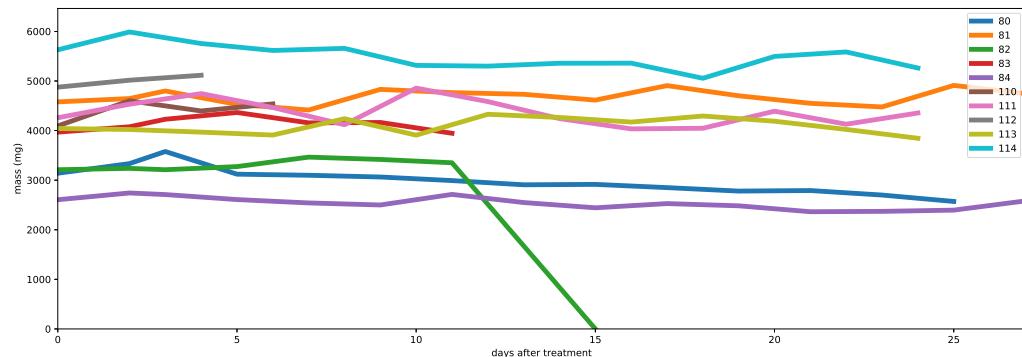


Table 1: Pairwise differences among mortality curves.

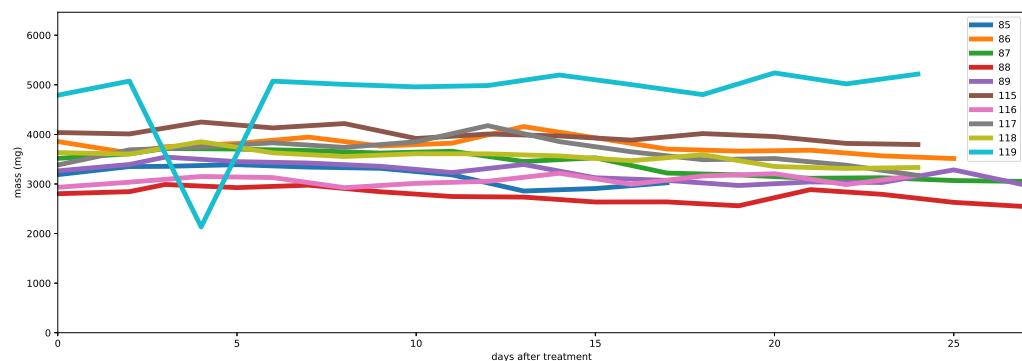
		test_statistic	p
control	heat inactivated	0.045611	0.830884
	virus	1.036616	0.308610
heat inactivated	virus	2.859672	0.090826

3 Mass

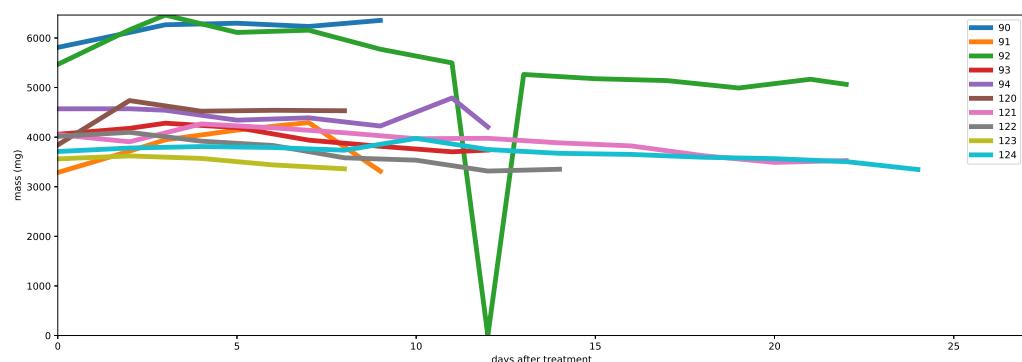
mass control.pdf



mass heat inactivated.pdf



mass virus.pdf



4 Postmortem Images

4.1 control



Figure 1: Bioassay: MALB-1; Treatment: control; Beetle ID: 80



Figure 2: Bioassay: MALB-1; Treatment: control; Beetle ID: 81



Figure 3: Bioassay: MALB-1; Treatment: control; Beetle ID: 82



Figure 4: Bioassay: MALB-1; Treatment: control; Beetle ID: 83



Figure 5: Bioassay: MALB-1; Treatment: control; Beetle ID: 84



Figure 6: Bioassay: MALB-2; Treatment: control; Beetle ID: 110



Figure 7: Bioassay: MALB-2; Treatment: control; Beetle ID: 111



Figure 8: Bioassay: MALB-2; Treatment: control; Beetle ID: 112



Figure 9: Bioassay: MALB-2; Treatment: control; Beetle ID: 113



Figure 10: Bioassay: MALB-2; Treatment: control; Beetle ID: 114

4.2 heat inactivated



Figure 11: Bioassay: MALB-1; Treatment: heat inactivated; Beetle ID: 85



Figure 12: Bioassay: MALB-1; Treatment: heat inactivated; Beetle ID: 86



Figure 13: Bioassay: MALB-1; Treatment: heat inactivated; Beetle ID: 87



Figure 14: Bioassay: MALB-1; Treatment: heat inactivated; Beetle ID: 88



Figure 15: Bioassay: MALB-1; Treatment: heat inactivated; Beetle ID: 89



Figure 16: Bioassay: MALB-2; Treatment: heat inactivated; Beetle ID: 115



Figure 17: Bioassay: MALB-2; Treatment: heat inactivated; Beetle ID: 116



Figure 18: Bioassay: MALB-2; Treatment: heat inactivated; Beetle ID: 117



Figure 19: Bioassay: MALB-2; Treatment: heat inactivated; Beetle ID: 118



Figure 20: Bioassay: MALB-2; Treatment: heat inactivated; Beetle ID: 119

4.3 virus



Figure 21: Bioassay: MALB-1; Treatment: virus; Beetle ID: 90



Figure 22: Bioassay: MALB-1; Treatment: virus; Beetle ID: 91



Figure 23: Bioassay: MALB-1; Treatment: virus; Beetle ID: 92



Figure 24: Bioassay: MALB-1; Treatment: virus; Beetle ID: 93



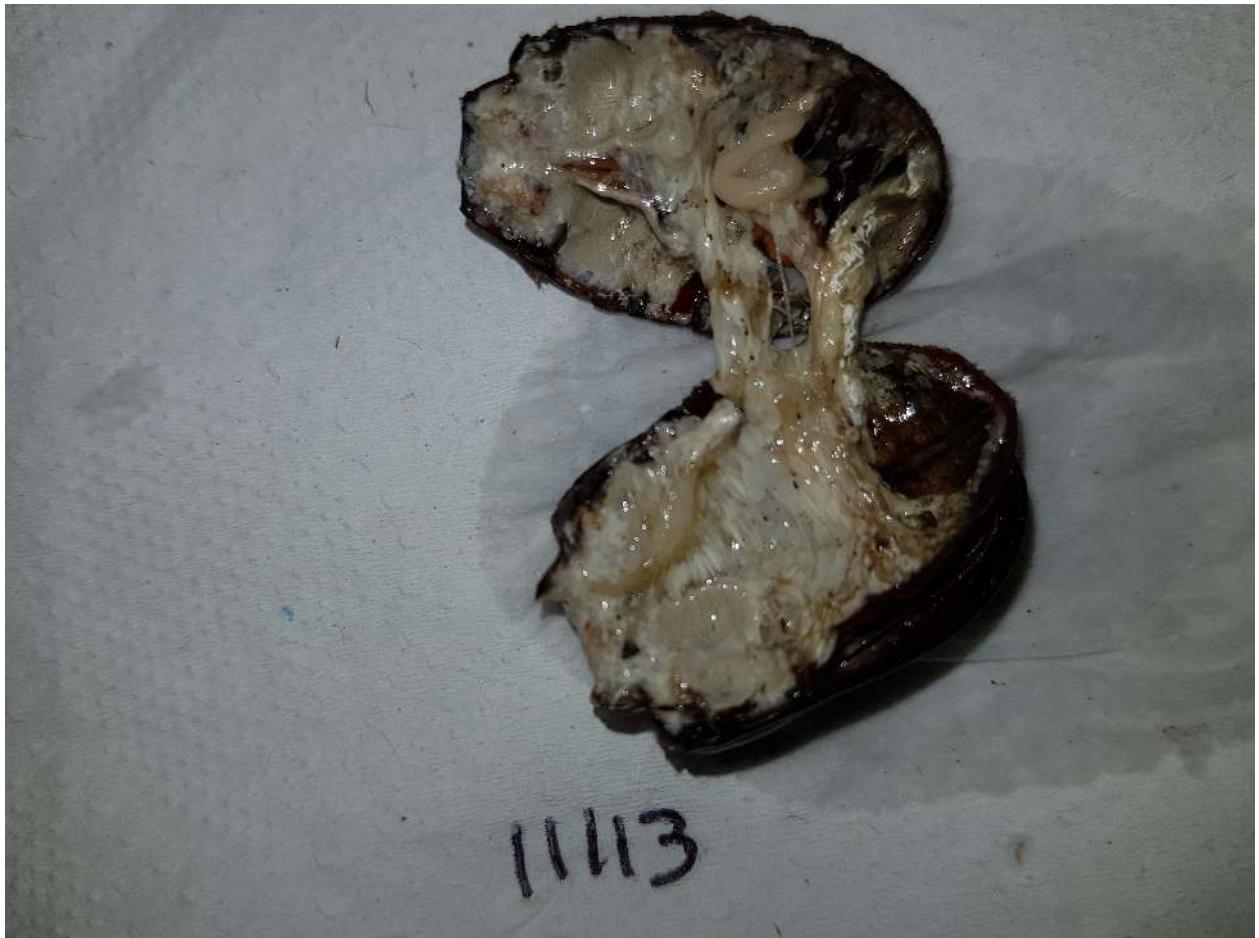
Figure 25: Bioassay: MALB-1; Treatment: virus; Beetle ID: 94



Figure 26: Bioassay: MALB-2; Treatment: virus; Beetle ID: 120



Figure 27: Bioassay: MALB-2; Treatment: virus; Beetle ID: 122



11113

Figure 28: Bioassay: MALB-2; Treatment: virus; Beetle ID: 123



Figure 29: Bioassay: MALB-2; Treatment: virus; Beetle ID: 124

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

See following page.



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PNG

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1 Description

Adult beetles incubated at 30°C and 80% RH for more than 2 weeks to observe possible contamination from green muscardine fungus infection were employed in a bioassay to determine the susceptibility of adults to infection by a virus isolate collected from Pupae New Guinea (**PNG**). Treatment 1 consisted of 10-20 µl sterile filtered water injected at a point on the ventral surface at the junction of the hind leg and the thoracic using a sterile needle. Treatment 2 consisted of 10-20 µl heat-inactivated virus injection while in the treatment 3, beetles were injected with 10-20 µl of untreated virus preparation. Adults were then placed in clean glass mason jars (bleach-treated) with a piece of banana added for food. Beetles were incubated at 30°C and 80% RH in a Percival incubator. All beetles were weighted every other day but monitored daily for four weeks to observe any possible signs of mortality.

1.1 Replicate 1

A total of seven adult females and eight adult males distributed among the three treatments were employed in this replicate.

1.2 Replicate 2

A total of six adult females and nine adult males distributed among the three treatments were employed in this replicate.

1.3 Replicate 3

A total of nine adult females and 12 adult males distributed among the three treatments were employed in this replicate.

1.4 Replicate 4

A total of 14 adult females and 16 adult males distributed among the three treatments were employed in this replicate.

2 Mortality

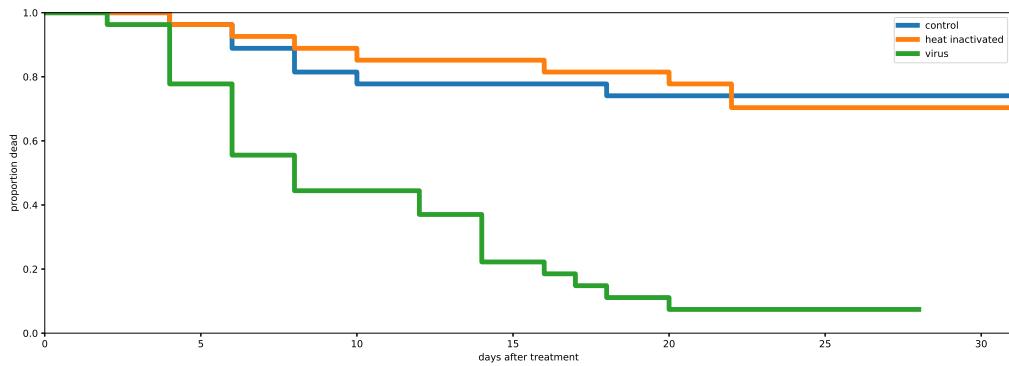
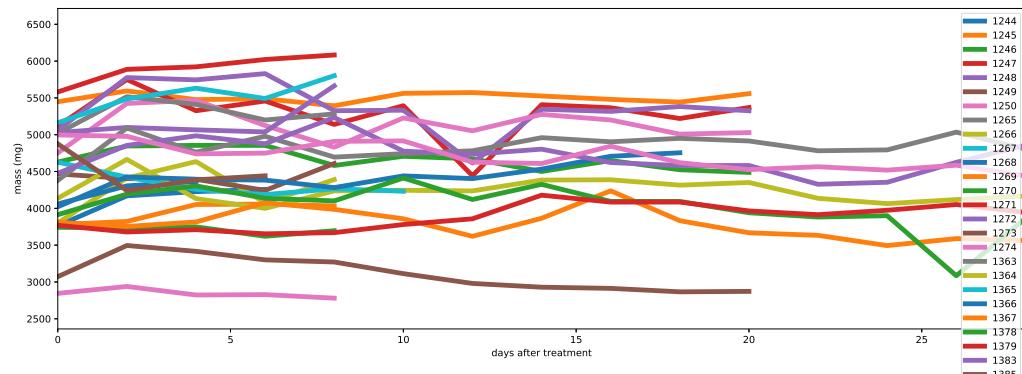


Table 1: Pairwise differences among mortality curves.

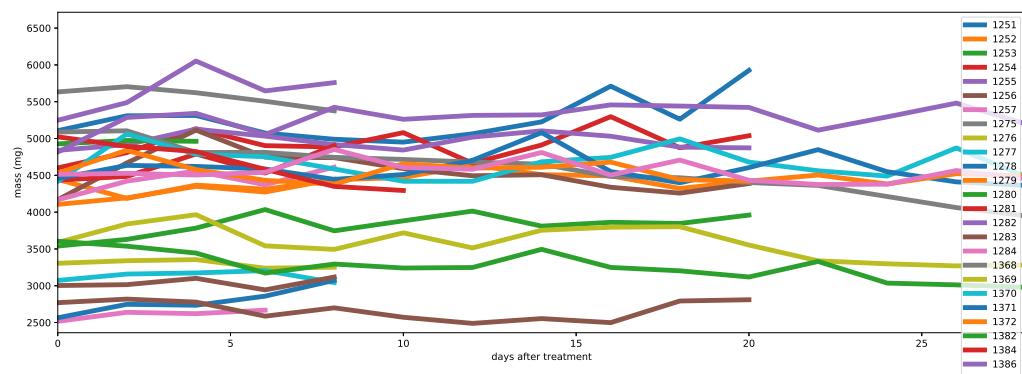
		test_statistic	p
control	heat inactivated	0.032268	8.574412e-01
	virus	25.286585	4.941336e-07
heat inactivated	virus	28.824370	7.924768e-08

3 Mass

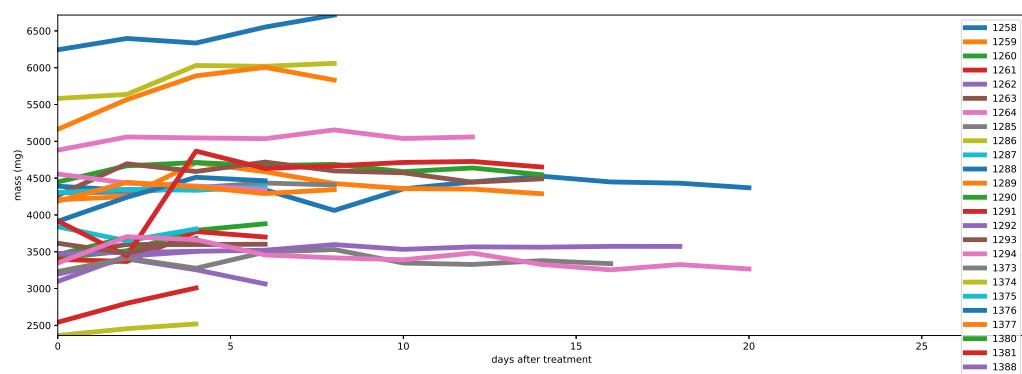
mass control.pdf



mass heat inactivated.pdf



mass virus.pdf



4 Postmortem Images

4.1 control



Figure 1: Bioassay: PNG-3; Treatment: control; Beetle ID: 1244



Figure 2: Bioassay: PNG-3; Treatment: control; Beetle ID: 1245



Figure 3: Bioassay: PNG-3; Treatment: control; Beetle ID: 1246



Figure 4: Bioassay: PNG-3; Treatment: control; Beetle ID: 1247



Figure 5: Bioassay: PNG-3; Treatment: control; Beetle ID: 1248



Figure 6: Bioassay: PNG-3; Treatment: control; Beetle ID: 1249



Figure 7: Bioassay: PNG-3; Treatment: control; Beetle ID: 1250

4.2 heat inactivated



Figure 8: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1251



Figure 9: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1252



Figure 10: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1253



Figure 11: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1254



Figure 12: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1255



Figure 13: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1256



Figure 14: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1257

4.3 virus



Figure 15: Bioassay: PNG-3; Treatment: virus; Beetle ID: 1258



Figure 16: Bioassay: PNG-3; Treatment: virus; Beetle ID: 1264



Figure 17: Bioassay: PNG-4; Treatment: virus; Beetle ID: 1289



Figure 18: Bioassay: PNG-4; Treatment: virus; Beetle ID: 1293

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

See following page.



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V23B

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4.3 control	47

1 Description

Adult beetles incubated at 30°C and 80% RH for more than 2 weeks to observe possible contamination from green muscardine fungus infection were employed in a bioassay to determine the susceptibility of adults to infection by a virus isolate collected from the Solomon Islands (**V23 B**). Treatment 1 consisted of 10-20 µl sterile filtered water injected at a point on the ventral surface at the junction of the hind leg and the thoracic using a sterile needle. Treatment 2 consisted of 10-20 µl heat-inactivated virus injection while in the treatment 3, beetles were injected with 10-20 µl of untreated virus preparation. Adults were then placed in clean glass mason jars (bleach-treated) with a piece of banana added for food. Beetles were incubated at 30°C and 80% RH in a Percival incubator. All beetles were weighted every other day but monitored daily for four weeks to observe any possible signs of mortality.

1.1 Replicate 1

A total of nine adult females and six adult males distributed among the three treatments were employed in this replicate.

1.2 Replicate 2

A total of 13 adult females and two males distributed among the three treatments were employed in this replicate.

1.3 Replicate 3

A total of seven adult females and eight adult males distributed among the three treatments were employed in this replicate.

1.4 Replicate 4

A total of seven adult females and 14 adult males distributed among the three treatments were employed in this replicate.

2 Mortality

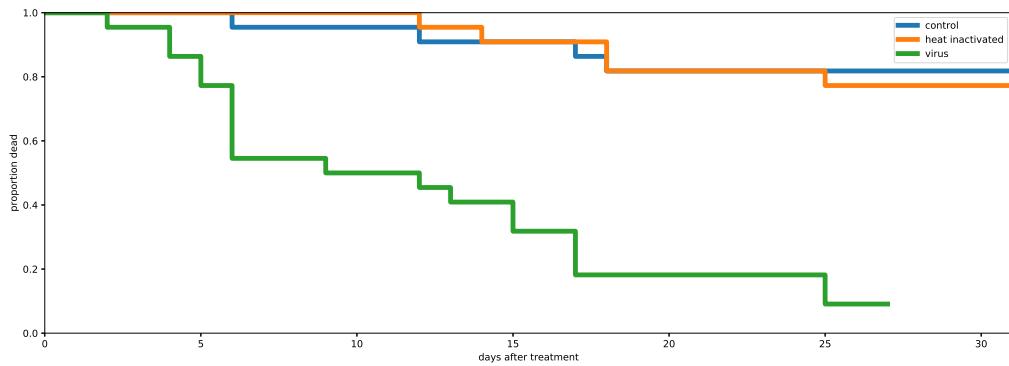
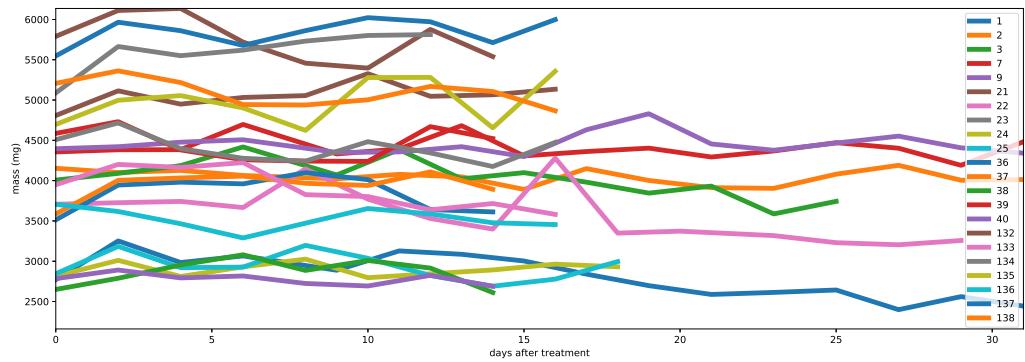


Table 1: Pairwise differences among mortality curves.

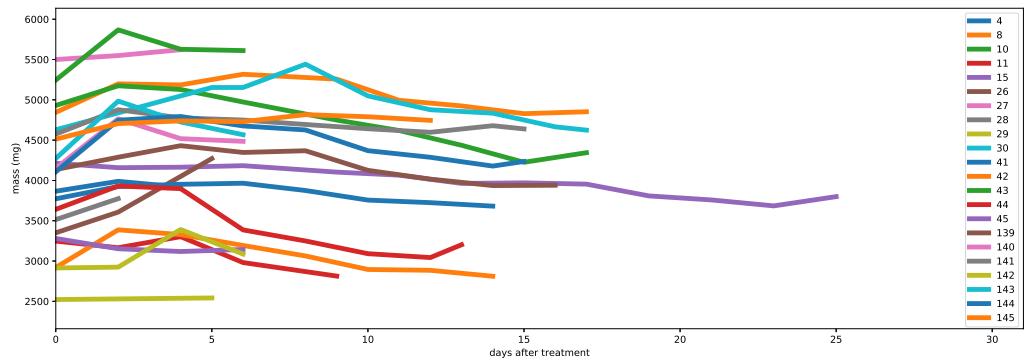
		test_statistic	p
control	heat inactivated	0.008648	9.259077e-01
	virus	26.455478	2.696826e-07
heat inactivated	virus	26.310731	2.906692e-07

3 Mass

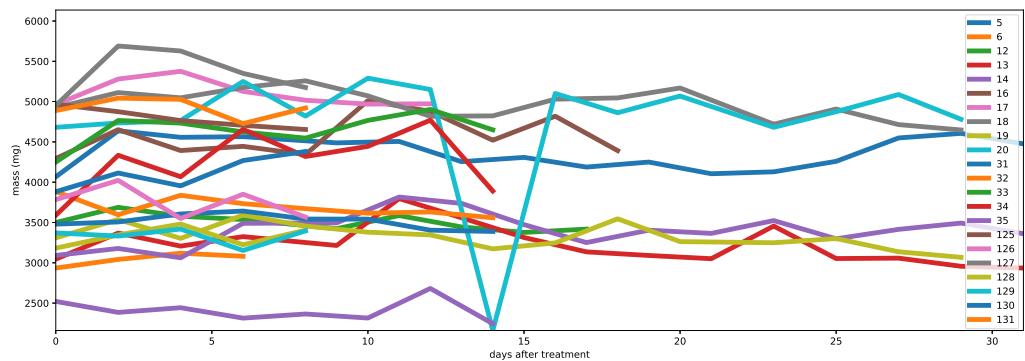
mass heat inactivated.pdf



mass virus.pdf



mass control.pdf



4 Postmortem Images

4.1 heat inactivated



Figure 1: Bioassay: V23B-1; Treatment: heat inactivated; Beetle ID: 1



Figure 2: Bioassay: V23B-1; Treatment: heat inactivated; Beetle ID: 2



Figure 3: Bioassay: V23B-1; Treatment: heat inactivated; Beetle ID: 3



Figure 4: Bioassay: V23B-1; Treatment: heat inactivated; Beetle ID: 7



Figure 5: Bioassay: V23B-1; Treatment: heat inactivated; Beetle ID: 9



Figure 6: Bioassay: V23B-2; Treatment: heat inactivated; Beetle ID: 22



Figure 7: Bioassay: V23B-2; Treatment: heat inactivated; Beetle ID: 23



Figure 8: Bioassay: V23B-2; Treatment: heat inactivated; Beetle ID: 24



Figure 9: Bioassay: V23B-2; Treatment: heat inactivated; Beetle ID: 25



Figure 10: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 36



Figure 11: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 37



Figure 12: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 38



Figure 13: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 39



Figure 14: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 40



Figure 15: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 132



Figure 16: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 133



Figure 17: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 134

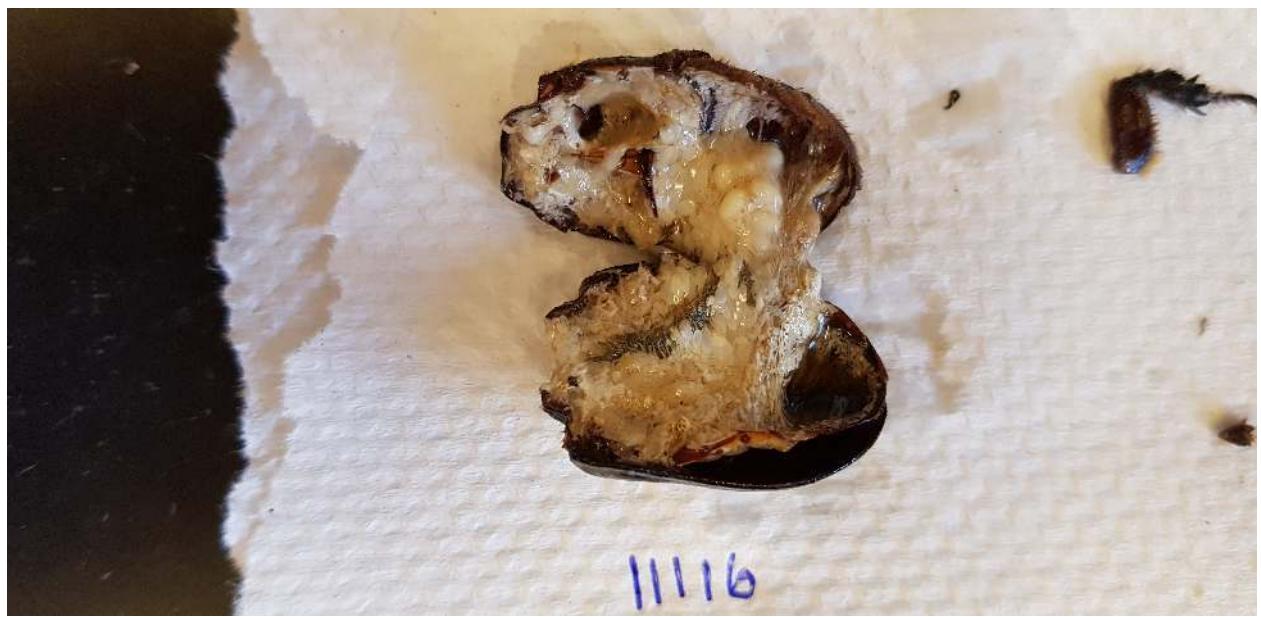


Figure 18: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 135



Figure 19: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 136



Figure 20: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 138

4.2 virus



Figure 21: Bioassay: V23B-1; Treatment: virus; Beetle ID: 4



Figure 22: Bioassay: V23B-1; Treatment: virus; Beetle ID: 8



Figure 23: Bioassay: V23B-1; Treatment: virus; Beetle ID: 10



Figure 24: Bioassay: V23B-1; Treatment: virus; Beetle ID: 11

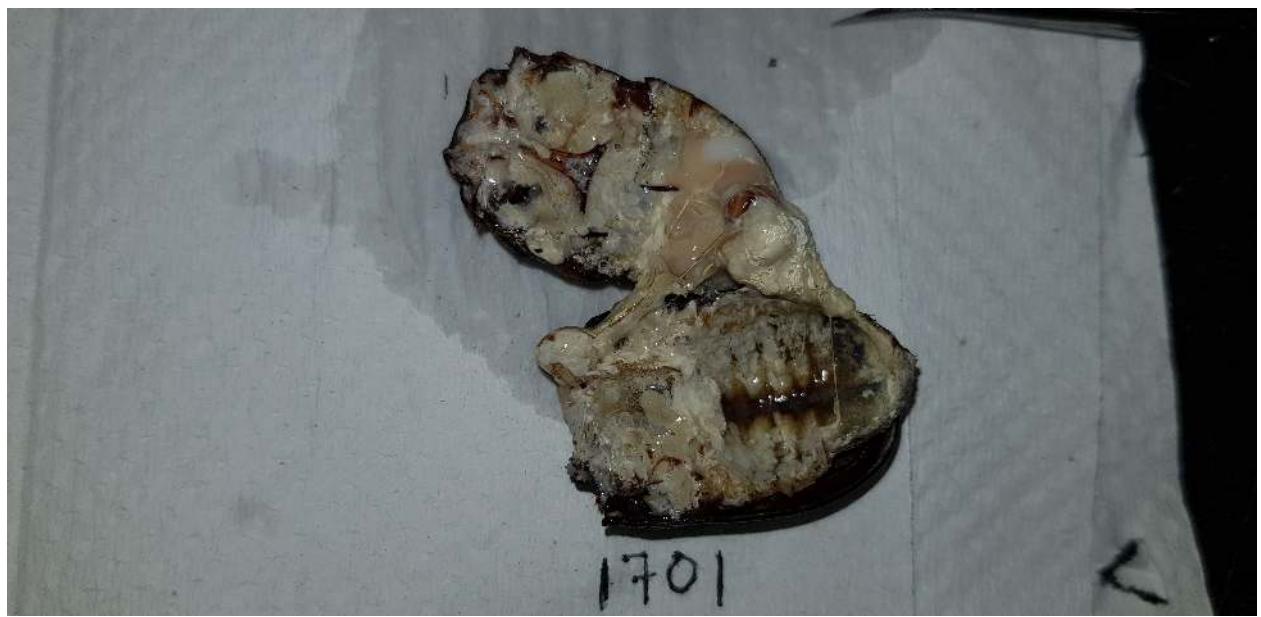


Figure 25: Bioassay: V23B-1; Treatment: virus; Beetle ID: 15



Figure 26: Bioassay: V23B-2; Treatment: virus; Beetle ID: 26



Figure 27: Bioassay: V23B-2; Treatment: virus; Beetle ID: 27

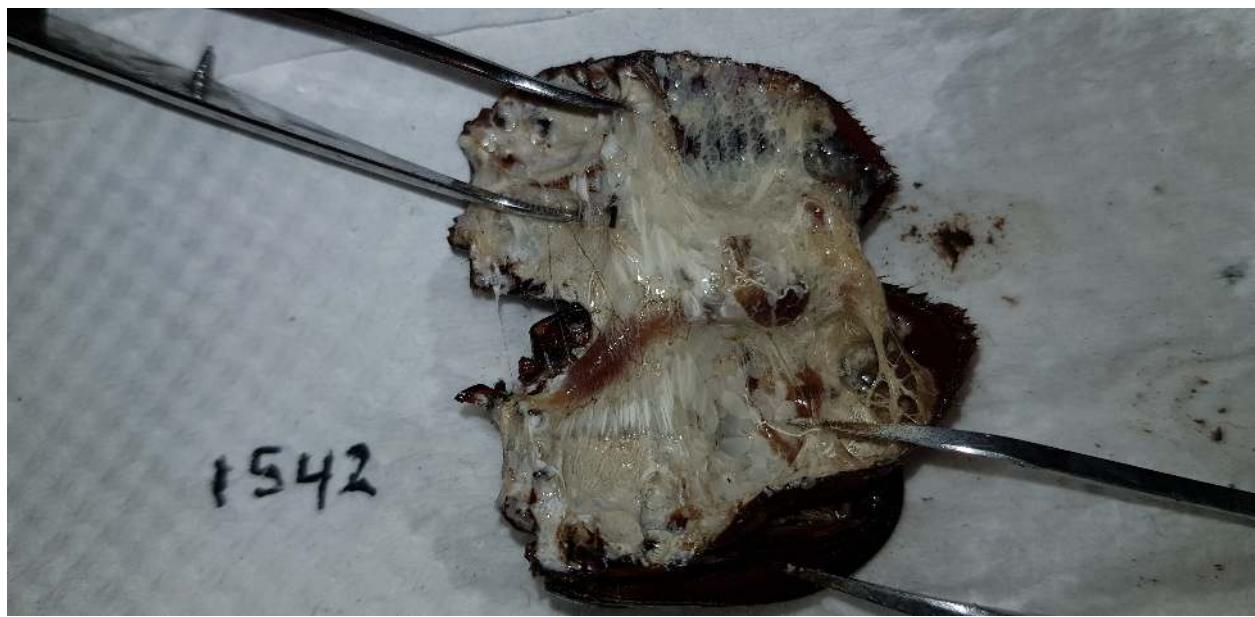


Figure 28: Bioassay: V23B-2; Treatment: virus; Beetle ID: 28



Figure 29: Bioassay: V23B-2; Treatment: virus; Beetle ID: 29



Figure 30: Bioassay: V23B-2; Treatment: virus; Beetle ID: 30



Figure 31: Bioassay: V23B-3; Treatment: virus; Beetle ID: 41



Figure 32: Bioassay: V23B-3; Treatment: virus; Beetle ID: 42



Figure 33: Bioassay: V23B-3; Treatment: virus; Beetle ID: 43



Figure 34: Bioassay: V23B-3; Treatment: virus; Beetle ID: 44



Figure 35: Bioassay: V23B-3; Treatment: virus; Beetle ID: 45



Figure 36: Bioassay: V23B-4; Treatment: virus; Beetle ID: 139



Figure 37: Bioassay: V23B-4; Treatment: virus; Beetle ID: 140

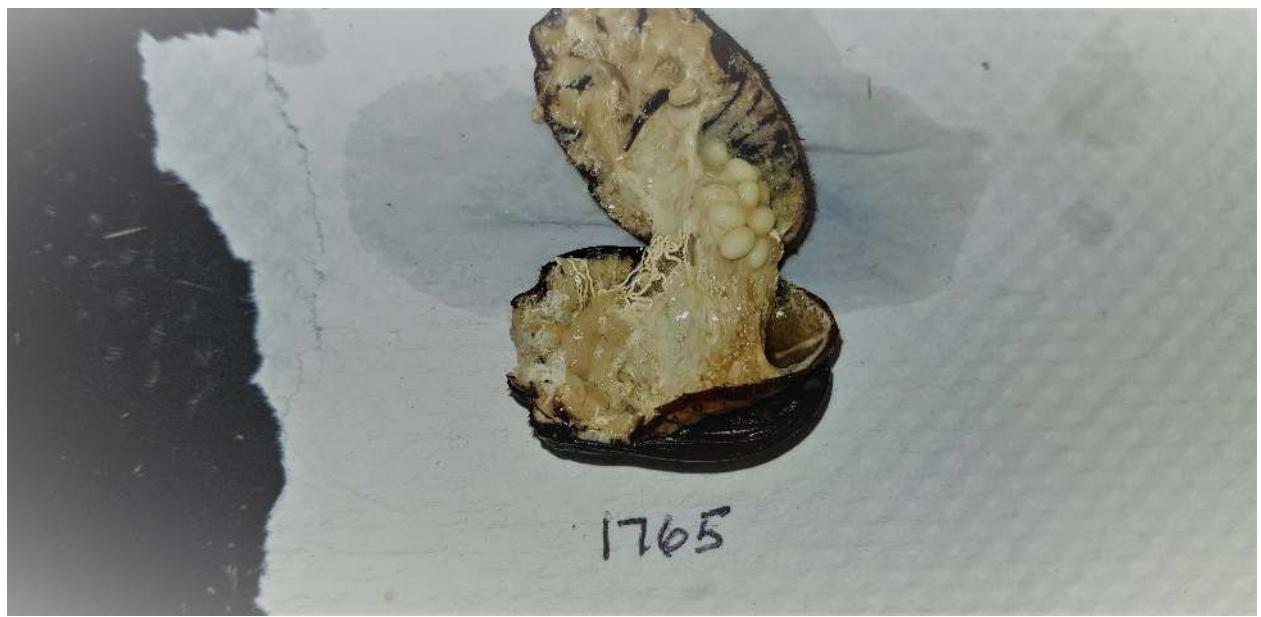


Figure 38: Bioassay: V23B-4; Treatment: virus; Beetle ID: 141



Figure 39: Bioassay: V23B-4; Treatment: virus; Beetle ID: 142

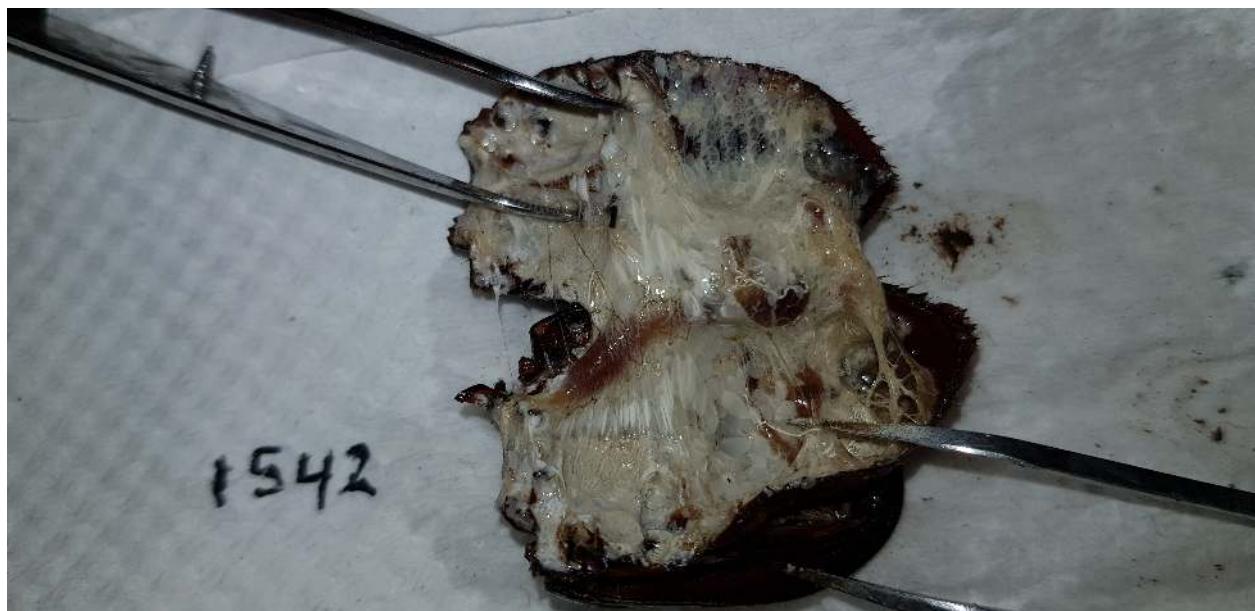


Figure 40: Bioassay: V23B-4; Treatment: virus; Beetle ID: 143



Figure 41: Bioassay: V23B-4; Treatment: virus; Beetle ID: 144



Figure 42: Bioassay: V23B-4; Treatment: virus; Beetle ID: 145

4.3 control



Figure 43: Bioassay: V23B-1; Treatment: control; Beetle ID: 5



Figure 44: Bioassay: V23B-1; Treatment: control; Beetle ID: 6



Figure 45: Bioassay: V23B-1; Treatment: control; Beetle ID: 12



Figure 46: Bioassay: V23B-1; Treatment: control; Beetle ID: 13

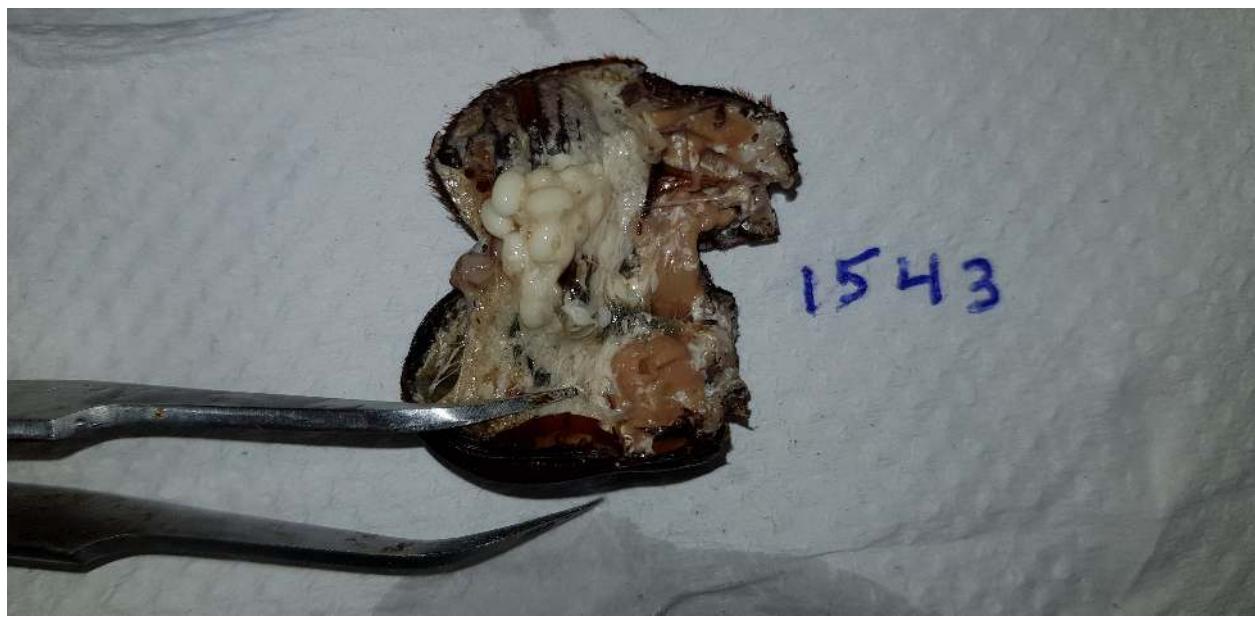


Figure 47: Bioassay: V23B-1; Treatment: control; Beetle ID: 14



Figure 48: Bioassay: V23B-2; Treatment: control; Beetle ID: 16



Figure 49: Bioassay: V23B-2; Treatment: control; Beetle ID: 17



Figure 50: Bioassay: V23B-2; Treatment: control; Beetle ID: 18



Figure 51: Bioassay: V23B-2; Treatment: control; Beetle ID: 19



Figure 52: Bioassay: V23B-2; Treatment: control; Beetle ID: 20



Figure 53: Bioassay: V23B-3; Treatment: control; Beetle ID: 31



Figure 54: Bioassay: V23B-3; Treatment: control; Beetle ID: 32



Figure 55: Bioassay: V23B-3; Treatment: control; Beetle ID: 33



Figure 56: Bioassay: V23B-3; Treatment: control; Beetle ID: 34



Figure 57: Bioassay: V23B-3; Treatment: control; Beetle ID: 35



Figure 58: Bioassay: V23B-4; Treatment: control; Beetle ID: 125



Figure 59: Bioassay: V23B-4; Treatment: control; Beetle ID: 126



Figure 60: Bioassay: V23B-4; Treatment: control; Beetle ID: 127



Figure 61: Bioassay: V23B-4; Treatment: control; Beetle ID: 128



Figure 62: Bioassay: V23B-4; Treatment: control; Beetle ID: 129



Figure 63: Bioassay: V23B-4; Treatment: control; Beetle ID: 130



Figure 64: Bioassay: V23B-4; Treatment: control; Beetle ID: 131