



University of Guam Coconut Rhinoceros Beetle Biological Control Project

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<https://github.com/aubreymoore/rearing3>

Bioassay Report: V23B

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<https://github.com/aubreymoore/rearing3/raw/master/bioassay-V23B.pdf>

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1 Summary

2 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.

2.1 Replicate 1

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

2.2 Replicate 2

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

2.3 Replicate 3

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

2.4 Replicate 4

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

3 Mortality

Table 1: Mortality summary.

| | bioassay_treatment | ntotal | ndead | mortality | adjusted_mortality | significance |
|---|--------------------|--------|-------|-----------|--------------------|--------------|
| 0 | control | 22 | 5 | 0.227273 | 0.000000 | 1.000000 |
| 1 | heat inactivated | 22 | 5 | 0.227273 | 0.000000 | 1.000000 |
| 2 | virus | 22 | 20 | 0.909091 | 0.882353 | 0.000009 |

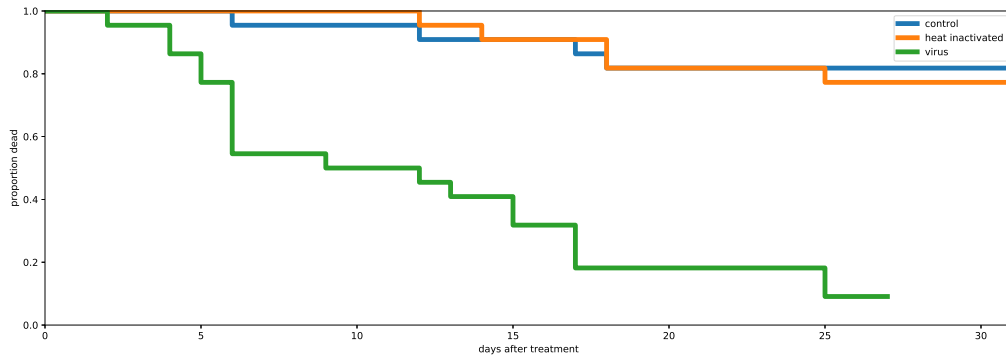


Table 2: Pairwise differences among mortality curves.

| | | 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 |

4 Change in Mass

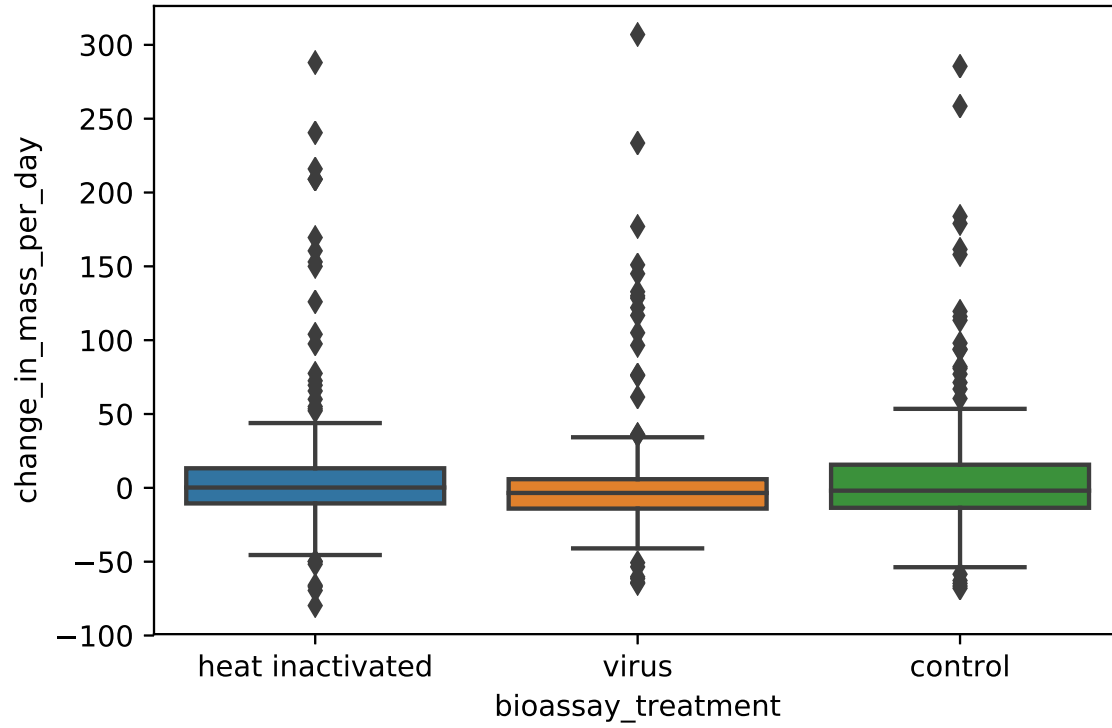


Table 3: Results of pairwise significance tests for differences in change in mass.

| | control | heat inactivated | virus |
|------------------|-----------|------------------|-----------|
| control | -1.000000 | 0.818527 | 0.818527 |
| heat inactivated | 0.818527 | -1.000000 | 0.707981 |
| virus | 0.818527 | 0.707981 | -1.000000 |

5 Post Mortem Images

5.1 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: 21



Figure 7: Bioassay: V23B-2; Treatment: heat inactivated; Beetle ID: 22



Figure 8: Bioassay: V23B-2; Treatment: heat inactivated; Beetle ID: 23



Figure 9: Bioassay: V23B-2; Treatment: heat inactivated; Beetle ID: 24



Figure 10: Bioassay: V23B-2; Treatment: heat inactivated; Beetle ID: 25



Figure 11: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 36



Figure 12: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 37



Figure 13: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 38



Figure 14: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 39



Figure 15: Bioassay: V23B-3; Treatment: heat inactivated; Beetle ID: 40



Figure 16: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 132



Figure 17: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 133



Figure 18: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 134



Figure 19: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 135



Figure 20: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 136



Figure 21: Bioassay: V23B-4; Treatment: heat inactivated; Beetle ID: 138

5.2 virus



Figure 22: Bioassay: V23B-1; Treatment: virus; Beetle ID: 4



Figure 23: Bioassay: V23B-1; Treatment: virus; Beetle ID: 8



Figure 24: Bioassay: V23B-1; Treatment: virus; Beetle ID: 10



Figure 25: Bioassay: V23B-1; Treatment: virus; Beetle ID: 11



Figure 26: Bioassay: V23B-1; Treatment: virus; Beetle ID: 15



Figure 27: Bioassay: V23B-2; Treatment: virus; Beetle ID: 26



Figure 28: Bioassay: V23B-2; Treatment: virus; Beetle ID: 27



Figure 29: Bioassay: V23B-2; Treatment: virus; Beetle ID: 28



Figure 30: Bioassay: V23B-2; Treatment: virus; Beetle ID: 29



Figure 31: Bioassay: V23B-2; Treatment: virus; Beetle ID: 30



Figure 32: Bioassay: V23B-3; Treatment: virus; Beetle ID: 41



Figure 33: Bioassay: V23B-3; Treatment: virus; Beetle ID: 42



Figure 34: Bioassay: V23B-3; Treatment: virus; Beetle ID: 43



Figure 35: Bioassay: V23B-3; Treatment: virus; Beetle ID: 44



Figure 36: Bioassay: V23B-3; Treatment: virus; Beetle ID: 45



Figure 37: Bioassay: V23B-4; Treatment: virus; Beetle ID: 139



Figure 38: Bioassay: V23B-4; Treatment: virus; Beetle ID: 140



Figure 39: Bioassay: V23B-4; Treatment: virus; Beetle ID: 141



Figure 40: Bioassay: V23B-4; Treatment: virus; Beetle ID: 142



Figure 41: Bioassay: V23B-4; Treatment: virus; Beetle ID: 143



Figure 42: Bioassay: V23B-4; Treatment: virus; Beetle ID: 144



Figure 43: Bioassay: V23B-4; Treatment: virus; Beetle ID: 145

5.3 control



Figure 44: Bioassay: V23B-1; Treatment: control; Beetle ID: 5



Figure 45: Bioassay: V23B-1; Treatment: control; Beetle ID: 6



Figure 46: Bioassay: V23B-1; Treatment: control; Beetle ID: 12



Figure 47: Bioassay: V23B-1; Treatment: control; Beetle ID: 13



Figure 48: Bioassay: V23B-1; Treatment: control; Beetle ID: 14



Figure 49: Bioassay: V23B-2; Treatment: control; Beetle ID: 16



Figure 50: Bioassay: V23B-2; Treatment: control; Beetle ID: 17



Figure 51: Bioassay: V23B-2; Treatment: control; Beetle ID: 18



Figure 52: Bioassay: V23B-2; Treatment: control; Beetle ID: 19



Figure 53: Bioassay: V23B-2; Treatment: control; Beetle ID: 20



Figure 54: Bioassay: V23B-3; Treatment: control; Beetle ID: 31



Figure 55: Bioassay: V23B-3; Treatment: control; Beetle ID: 32



Figure 56: Bioassay: V23B-3; Treatment: control; Beetle ID: 33



Figure 57: Bioassay: V23B-3; Treatment: control; Beetle ID: 34



Figure 58: Bioassay: V23B-3; Treatment: control; Beetle ID: 35



Figure 59: Bioassay: V23B-4; Treatment: control; Beetle ID: 125



Figure 60: Bioassay: V23B-4; Treatment: control; Beetle ID: 126



Figure 61: Bioassay: V23B-4; Treatment: control; Beetle ID: 127



Figure 62: Bioassay: V23B-4; Treatment: control; Beetle ID: 128

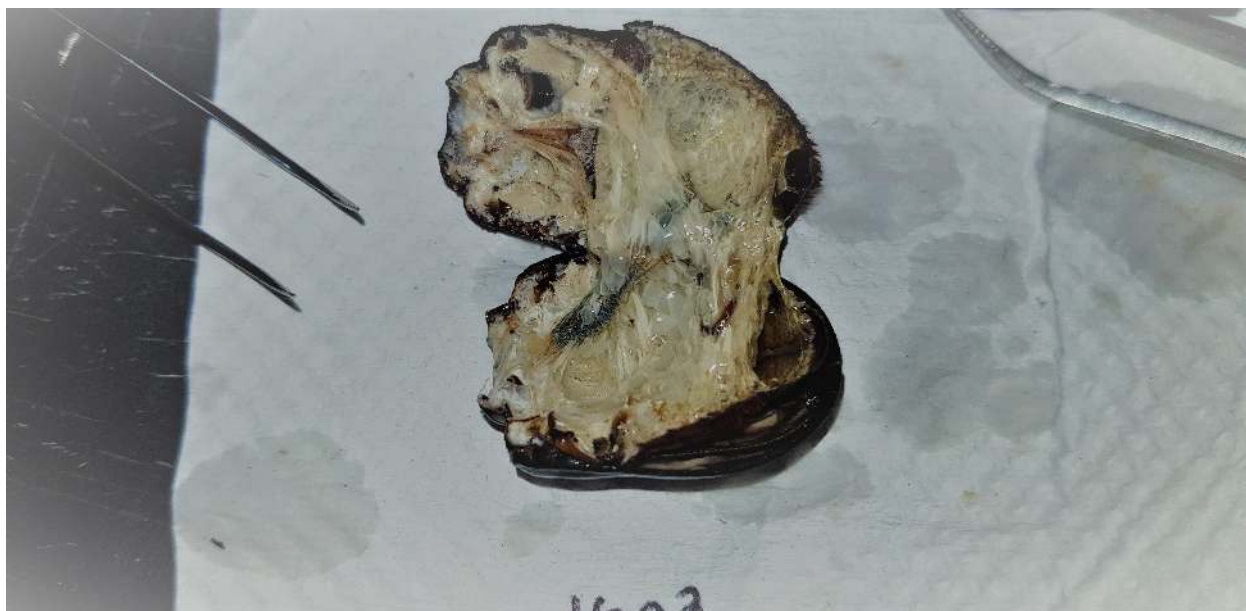


Figure 63: Bioassay: V23B-4; Treatment: control; Beetle ID: 129



Figure 64: Bioassay: V23B-4; Treatment: control; Beetle ID: 130



Figure 65: Bioassay: V23B-4; Treatment: control; Beetle ID: 131