

DRAFT

Proposed Method for Dosing Coconut Rhinoceros Beetle Adults with OrNV

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1 Introduction

Several methods have been used to dose coconut rhinoceros beetle adults (CRB) with *Oryctes rhinoceros* nudivirus (OrNV):

| Method | Pros | Cons |
|--|---------------------------|--|
| Application of droplets containing OrNV particles to mouthparts | Requires very little OrNV | Amount ingested may be highly variable |
| Forcing CRB to swim in an aqueous suspension containing OrNV particles | | Requires a large amount of OrNV |
| Direct injection into the hemocoel | Unatural | Allows for precise dosing. Requires a very small amount of OrNV. |

We propose an alternative dosing method which models feeding behavior of CRB adults within bore holes. A CRB adult usually initiates boring behind a petiole. The beetle typically bores horizontally into the center of the crown-shaft and then bores downwards. The beetle feeds on sap when exudes from tissue which is macerated during the boring activity.

In our dosing technique, we confine a beetle to the bottom of a disposable centrifuge tube (details) which models the vertical portion of a bore hole (Fig. 1). Before introducing a beetle, 2 ml of 5% sucrose solution containing 10^6 infectious units of OrNV are added to the tube. This liquid models sap from which collects at the bottom of the vertical section of a borehole.

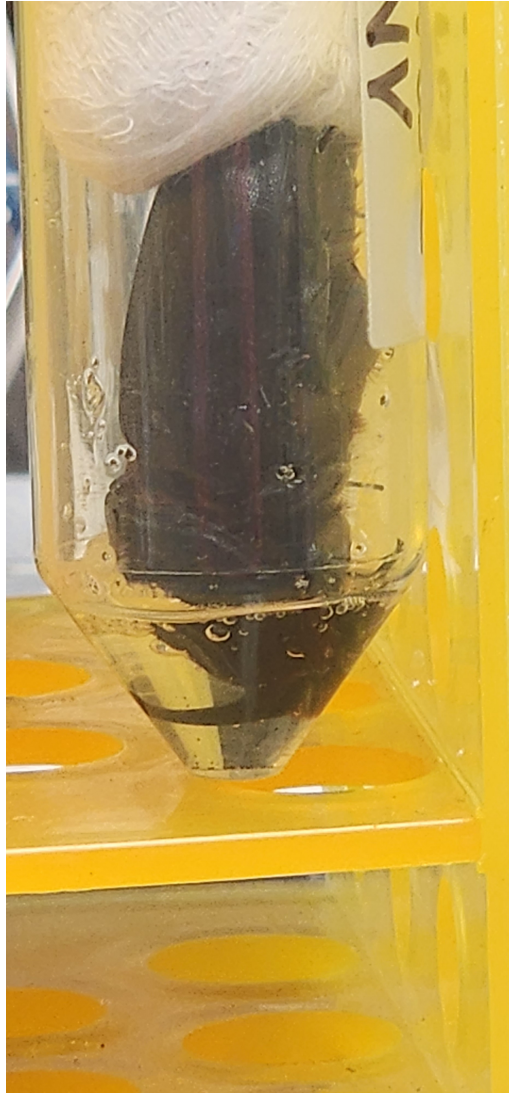


Figure 1: CRB adult being dosed in a centrifuge tube. Mouthparts are submerged in 2 ml of 5% sucrose solution containing 10^6 infectious units of OrNV for 15 minutes. A wad of cheese cloth above the beetle keeps it in place.

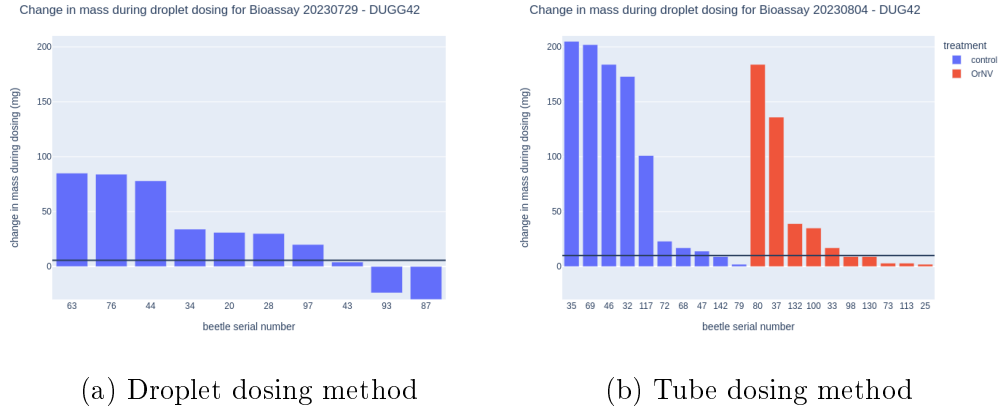


Figure 2: Black horizontal lines indicate mass containing 5,000 infective units (UI) of OrNV. This level is the the minimum dose recommended to establish infection in a susceptible beetle (AgResearch New Zealand 2023).

2 Notes

1. Our technique might work better if we increase sugar content (currently 5%). Sugar content of coconut sap is 12.92% (6.91% sucrose, 3.48% fructose, and 2.53% glucose) (Asghar et al. 2019).

3 References

AgResearch New Zealand (2023). *Unpublished report: Preparation of OrNV solution for delivery (applies to virulence/screening bioassays or transmission bioassays).*

Asghar, Muhammad Tuseef, Yus Aniza Yusof, Mohd. Noriznan Mokhtar, Mohammad Effendy Ya'acob, Hasanah Mohd. Ghazali, Lee Sin Chang, and Yanty Noorzianna Manaf (Sept. 30, 2019). "Coconut (Cocos Nucifera L.) Sap as a Potential Source of Sugar: Antioxidant and Nutritional Properties". In: *Food Science & Nutrition* 8.4, pp. 1777–1787. ISSN: 2048-7177. DOI: [10.1002/fsn3.1191](https://doi.org/10.1002/fsn3.1191). pmid: [32328243](https://pubmed.ncbi.nlm.nih.gov/32328243/). URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7174220/> (visited on 08/05/2023).