Experimental Plan: Determining Presence of OrNV in the Guam CRB-G Population

Objective

Methods
- Pheromone Traps
- Frass Samples
- Dissection
- PCR
- Histology
- Follow-up Activities

References

Objective

- The objective is to provide conclusive evidence for presence or absence of OrNV infection in the wild CRB-G population on Guam.
- OrNV was previously tentatively detected in CRB collected in pheromone traps at Leo Palace and the Yigo Agricultural Experiment Station.
- We are not trying to measure incidence (proportion of beetles infected).

Methods

Pheromone Traps

- We will collect CRB specimens from ?? panel traps already deployed at Leo Palace and 31 barrel traps deployed at the Yigo Agricultural Experiment Station. We will collect 100 CRB from each location.
- Traps will be baited with ChemTica oryctalure and will be visited every 2 or 3 days. Each beetle will be placed in a new sample jar (pottle).
- EQUIPMENT:
- DATA: trap number, trap gps location, number of CRB caught, number taken for tissue sampling (where possible collection of live beetles is preferable)

Frass Samples

- Beetles will be kept in pottles for at least 24 hours. Immediately after beetles are remove for dissection, the pottle will be washed be adding 1 ml of sterile distilled water. Following vigorous shaking ?? microlitres will be pipetted out of the pottle and processed by PCR to detect OrNV.
- EQUIPMENT:
- DATA:

Dissection

- Gut samples will excised and processed using standard protocol (1) (REF)
- Samples will be split for OrNV detection at UOG and at AgResearch
    - Tissue for Histology: after head is removed, take first 1cm of gut tissue (anterior midgut) and place into histology preservative (FAA or 10% formalin=neutral buffered 4% formaldehyde). Store at room temperature for at least 24h before processing . Ship to NZ at room temperature is ok. Do NOT freeze!
    - Tissue for PCR: following dissection and preservation of anterior midgut, remove 0.5-1cm of tissue and place into PCR preservative (monopropylene glycol, or 95+% ethanol). Additionally remove 4-6 legs and place into a new tube (all legs can go together, just separate from gut tissue). Store tissue at -20C until ready to extract DNA. Ship to NZ at room temperature is ok.
- EQUIPMENT: for each individual: 1x large scissor (cut off head), 1x fine forceps (to open body cavity, cut pieces of gut tissue), 2x fine forceps (tease out gut tissue, move organs around as necessary). Lighter, bunsen burner or alcohol burner plus ethanol or methylated spirits to flame tools. Lots of paper towels to clean tools and contain dissection debris for each individual dissected. Tubes prefilled
- DATA: for each individual record gross visual observations (gut is white/brown, thin/thick, gender, date of dissection, preservatives used, etc). Ensure that collection information can be traced back for each individual beetle.

DNA extraction and PCR

- We need to test gut samples for presence of OrNV and to determine CRB biotype (CRB-G or CRB-S)
- DNA extraction:
    - From CRB Gut tissue: follow standard tissue gDNA protocol from chosen kit. Elute in 50ul of buffer. (for spin column I tend to do 2 extractions... 1st with ~25ul, 2nd with 25-50ul... there is always some loss of volume hence the little bit of extra).
        - From extracted gDNA, make 1:100 and 1:5000 dilutions of DNA for use as PCR templates.
        - Follow PCR protocol (we use the 15a/15b primer set, as this has worked well for us and correlates with histology results
    - From CRB Frass solution: follow standard liquid gDNA protocol from chosen kit.
        - Note that it may be necessary to optimize extraction method for frass (e.g. concentrate raw solution etc)
        - Pilot test a PCR DNA template dilution series (e.g. undiluted, 1:10, 1:100, 1:1000, 1:5000/1:10000). This can be compared back to tissue DNA but also as an independent method use histology results to check likelihood of virus infection.
        - Follow PCR protocol (we use the 15a/15b primer set, as this has worked well for us and correlates with histology results)
- PCR:
- EQUIPMENT:
- DATA: record observations from agarose gel (band present, weak band, absent) and interpretation of virus infection likely or not (based on dilution of template).

Histology

- Ship histology preserved specimens to NZ for analysis. Use a formaldehyde based preservative (FAA works well, but if not available 10% formalin= neutral buffered 4% formaldehyde).
    - Standard histology methods will be used. Tissue will be paraffin embedded, sectioned, and stained (Hemotoxylin and Eosin; H&E), coverslips mounted and slides examined for status of health (i.e. look for symptoms of virus infection).
- EQUIPMENT: compound microscope capable of 100x and 400x magnification for analysis of histology slides.
- DATA: record microscopy observations and interpretation of virus infection likely or not.

Follow-up Activities

- If OrNV is detected we will need to do the following:.
- a lab bioassay to test Koch's postulate
- characterize it to try and figure out if it matches the virus we released years ago or if it is likely to be a fortuitous introduction.
- Do island-wide surveys to determine how widely the virus is spread.
- Determine if the virus is having reducing CRB damage on Guam. If so, maybe we should move ahead to propagate it and do autodissemination in areas where incidence is low.
- Share info and virus samples with collaborators in the CRB-G action group.

References

1. Marshall SDG, Moore A, Vaqalo M, Noble A, Jackson TA. A new haplotype of the coconut rhinoceros beetle, *Oryctes rhinoceros*, has escaped biological control by *Oryctes rhinoceros* nudivirus and is invading Pacific Islands. J Invertebr Pathol [Internet]. 2017 Oct 1 [cited 2017 Aug 26];149:127–34. Available from: http://www.sciencedirect.com/science/article/pii/S0022201117300289