

Progress in Bioassays of OrNV Isolates to Detect Biocontrol Candidates for CRB-G

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I. In vivo analysis of V23B OrNV isolate

A laboratory bioassay was conducted employing 54 adult beetles divided into either an untreated control group with each beetle fed 20 μ l sterile water and a virus-treated group with each beetle fed 20 μ l virus preparation. To compare the control with virus treatment, a mean was calculated for each of two consecutive weights from individual beetles taken every 2 days during a four-week test period to determine the effect of virus on the beetle. A relative weight loss difference was calculated based on the mean of the initial beetle weight at start of the test minus its final mean weight at the end of the test period divided by the beetle initial weight (Fig. 1). There was no significant difference in weight loss between the untreated control and virus-treated group based on a two-sample t-test assuming unequal variances. These results indicate that relying on a weight measurement may not be a reliable method to determine the actual impact of the virus on the morbidity of the insect.

Fig.1

Table 1. Mean loss of weight (mg) in adult CRB under two treatments

	Control	Virus-treated
Mean	0.09070833	0.08185
	3	
Variance	0.00309517	0.00216630
	2	7
Observations	24	16
Hypothesized Mean Difference	0	
df	36	
t Stat	0.54482172	
	5	

II. In vivo analysis of CRB samples collected from Taiwan.

Coconut rhinoceros beetle adults were collected from two geographical regions in Taiwan: (1) the Taichung Co., Wufeng District in western-central Taiwan and (2) Kaohsiung City, Dashu District in southern portion of the country. All 80 beetles were dissected to determine any possible viral infection as visually indicated by a whitish-swollen midgut. Unfortunately, none of the midgut showed any outward signs of infection. However, the midgut from each dissected sample was then further split into two subsamples - one for DNA analysis while the remaining portion for bioassay testing. In the bioassay tests, beetles collected on Guam and maintained for over two weeks prior to the test were orally infected with from 10 – 20 µl of virus prepared from crushed Taiwan beetle midguts. Of the 90 beetles tested only 4 (4.5%) showed possible virus presence. These positive samples were dissected and maintained at 4°C for later PCR analysis.

III. Establishment of a molecular biology laboratory for PCR studies

We have almost completed setting up of a fully functional laboratory designed to conduct PCR analysis of genomic DNA from both viral and CRB samples. This will enable us to detect more definitively along with any accompanying *in vivo* bioassays the presence of OrNV in the insect.

IV. Experiments to establishment of a continuous insect cell line from the coconut rhinoceros beetle (CRB), *Oryctes rhinoceros* (L.).

Several Coleopteran cell lines have been previously established for *in vitro* studies to evaluate the potential application of entomopathogenic viruses as a biocontrol agent. However, these cell lines, which have been used in the laboratory production of various *Oryctes rhinoceros* virus isolates, are non-homologous cell lines (i.e. not derived from CRB) and may not reflect the actual potential infectivity and production level that this virus is capable of attaining in its insect of origin. One such non-homologous cell line derived from the African black beetle, *Heteronychus arator* (F.) (HA) has been cultured for decades for use in the *in vitro* production of various OrNV isolates. Several issues of concern have been voiced in the literature to indicate that HA may not be the most ideal cell culture system for the mass production of infectious and viable ORNV isolate(s). Consequently, an attempt was made to dissect midgut tissue from adults and larvae to obtain cell culture material; however, both have large amounts of undigested and extraneous material in their system which presents a challenge to avoiding contamination so the focus was then to extract hemolymph tissue (a “cleaner” part of the insect) from a range of the different larva stages (from early 2nd to late 3rd instars). So far attachment of cell tissue has been observed in two cell culture media tested. However, to obtain a stable, continuously growing CRB cell line will require a lengthy cell incubation period before actual *in vitro* tests of the different OrNV isolates can be conducted.