Report of research consultancy to Guam for implementation of virus control of invasive coconut rhinoceros beetle (CRB)

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Overview and recommendations

Good progress has been made in containing the coconut rhinoceros beetle (CRB) outbreak on Guam. Spread has been slow and numbers are not high in relation to other reported outbreaks. It is still possible to eradicate the beetle from Guam and successful introduction of the *Oryctes* virus will play an important role in this process. General comments on the outbreak and virus status of CRB on Guam;

- The situation of Guam is unique as a transport hub with the danger of providing sources of beetles to other un-infested Pacific states and to the American mainland. Failure to eradicate the insect will require ongoing high quarantine costs.
- In the outbreak hot spots, the level of damage for the number of beetles collected is very high in comparison with other beetle infested regions.
- Beetles collected from the pheromone traps showed no symptoms of virus infection and some females were carrying high numbers of eggs.
- Beetles maintained in captivity can be very long-lived and can continue feeding for several months.
- Laboratory analysis by PCR also shown no evidence of virus in the established population. A further twenty samples have been taken from beetles captured in pheromone traps from different parts of the island.

These observations all support the hypothesis that the Guam population of CRB is *Oryctes* virus free. Procedures for use of the virus must be considered carefully as this is the first time the virus has been used to assist the eradication of an outbreak of CRB.

Introduction of *Oryctes* virus will weaken the established population by reducing vigour and fecundity. The virus does not appear to have established from the initial release in September 2009. Further scientific support (for example a Post Doctoral researcher) is necessary to provide detailed experimentation and implementation to ensure success of this part of the programme. Suggestions for virus introduction and establishment;

- Virus infection and release should be limited to male beetles. In the initial phase these can be collected, or reared from immature stages, from breeding sites.
- Female beetles should be used for laboratory studies to confirm pathology, infectivity and transmission.
- The virus release programme and confirmation of virus infectivity and pathology should be carried out concurrently as establishment of the virus in the outbreak population is an important priority.
- Success of the infected male release programme should be assessed by recapture of marked adults and detection of transmission through PCR detection of infected beetles from pheromone trap catches.
- Questions relating to confirmation of pathology, infectivity of the current biocontrol and alternative strains, and impact on feeding and fecundity, should be carried out in the contained laboratory with female beetles from collection or the rearing facility.
- Suggestions to assist the infection and rearing programmes are contained in this document.
- Virus infected adult male beetles should be primarily released in the main outbreak areas with highest density of beetles. A systematic plan for release should be developed.
- Development and management of the virus implementation programme requires detailed scientific experimentation, evaluation and management to get the best results as quickly as possible. Appointment of a Post Doctoral researcher for a two year contract would greatly enhance the chance of successful eradication.
- The virus introduction programme should be reviewed in 12 months to develop its role in population eradication or suppression.

The CRB outbreak in Guam has some unique characteristics which must be taken into consideration for the eradication of CRB from Guam.

- Beetles were found to be developing in unmanaged palms with high organic matter accumulation in the frond axils and crowns. This situation appears to be unique to Guam. It is recommended to characterise potential crown breeding sites (based on organic matter accumulation), cut and clear these palms from unused sites and reduce the potential contribution to the CRB populations from palms in recreational areas by regular management and removal of fronds.
- Decaying branches of trees in the target zones should be investigated as potential breeding sites. Decaying flame tree branches were found to show evidence of CRB feeding and development.

- The impact of the sanitation programmes should be assessed by implementation of a regular damage survey of marked palms. Assessment of damage to the four top fronds allows recent damage to be monitored.
- Palm removal will leave stumps in the ground that can become breeding sites for the beetles. These should be regularly monitored by the dog teams. The use of lignin degrading fungi should be investigated to advance breakdown of the root bole and reduce available food resource for CRB larvae.

General review

The objective of this consultancy has been to introduce the biocontrol agent *Oryctes* virus to assist the programme for eradication of CRB on the island of Guam. CRB is native to SE Asia and Sth Asia where its damage to palms is well known. For the last 100 years it has been an invasive pest in the Pacific starting in Samoa in 1912 and spreading to other islands later in the century. In all cases damage from the invasive wave and density of beetles has been much higher than in their native habitat raising the obvious question that the invasive insects have escaped from their natural predators and pathogens and have been able to reach unprecedented population densities and cause enormous damage to palms in the new homeland. For example, Gressit 1953 recorded 50% mortality of coconut palms in Palau after beetle invasion in the 1940's. A search for natural enemies of CRB in Asia in the 1960's led to the discovery of *Oryctes* virus which was widespread among beetle populations in Malaysia (reviewed by Huger 2005). Introduction of the virus into the Pacific was followed by a dramatic decline in beetle numbers (reviewed in Jackson 2009) and once introduced the virus appears to be maintained in the affected populations as a persistent infection causing morbidity and lack of vigour in the CRB population.

CRB was first recorded from Guam in 2007 and follow-up surveys showed that the pest was well established at Tumon Bay and Faifai beach. A collaborative programme by University of Guam and USDA to eradicate the pest was rapidly initiated and surveys and sanitation, and chemical control actions have been carried out in attempts to eliminate the pest. Regular monitoring of the pest with pheromone traps has shown that the pest is still present but the spread of the insect appears to have been limited in comparison to other islands invaded by the pest.

This consultancy has provided the opportunity to compare CRB populations and damage with those of other infested islands. The most striking element of the CRB invasion on Guam is the very high level of damage to coconut palms that can be observed in the outbreak areas. This is even more striking when pheromone trap catches are added which suggest that the flying population is much more damaging than that in other infested states.

In the outbreak zone of Tumon most palms are showing extensive damage and a survey based on visual damage of the upper four fronds (a standardised method of assessment in the Sth Pacific) indicates close to 100% of palms damaged. Pheromone trapping in the Tumon zone indicates a beetle catch of 0.006 per trap per day (Moore, survey data). In contrast, Samoa where average damage is 30% of palms showing feeding injury (Data from MAF, Samoa) the average trap catch is 0.15 beetles per trap per day (25 x greater).

	
Coconut palm attacked by CRB on the Tumon waterfront	CRB adult boring into the growing palm shoot
	
Crown of a heavily damaged palm typical of the Tumon infestation area	A damaged palm typical of attack on Upolu, Samoa
0.006	0.154
Beetles caught per pheromone trap, per day in Tumon	Beetles caught per pheromone trap, per day in Upolu, Samoa

Beetle damage in Guam tends to be highest in areas of old unmanaged palms. A visual survey of the old Fujita hotel site in Tumon indicated high levels of fresh damage despite a significant sanitation programme in the area, few beetle catches in the pheromone traps and regular searches by the beetle team unable to find breeding sites on the ground. Unmanaged palms in the hotel site had high amounts of dead and decaying organic matter in their crowns produced by old palm fronds and fruiting bunches. This material was considered to have the potential for CRB larval development. Working with the beetle team to cut down palms it was soon revealed that immature CRB stages eggs and larvae were present in the unmanaged palm tops and that this represents a significant source of fresh beetle adults in these areas. Managed palms, where fronds and fruits are removed, seem to offer little opportunity for larval development and severe damage appears very localised, within and close to the unmanaged areas. Interestingly in hotel landscapes where coconut palms are

trimmed there is little CRB damage evident except in palms adjacent tounmanaged areas.

CRB development in live standing palms is unknown or unreported in other infested countries. This special characteristic of the Guam outbreak should be studied further to allow the beetle team to identify the characteristics of high risk palms and manage them accordingly. As a first step the unmanaged palms in the undeveloped areas could be removed or at least trimmed to remove the food source for beetle development. Rotting tree trunks of other species can also provide sites for beetle development. In particular rotting branches of the flame tree should be checked for larvae and removed if found susceptible.

Unmanaged palm showing organic matter build up in the crown

Managed palm with little organic material in the crown

Third instar beetle larva found feeding on rotting frond bases in the palm crown

Large 3rd instar CRB larvae extracted from the crown of coconut palm, Tumon area

The first attempt to introduce the virus in September 2009 appears to have failed. This will be confirmed from current sampling. There are a variety of possible reasons but none can be conclusively established from retrospective sampling. The objective of Phase 2 of the project is to conclusively establish infection in Guam CRB and establish monitoring of virus spread in the field. A new virus shipment has been produced and imported from AgResearch and the infection method and assessment systems modified. Most virus releases in the past have been aimed at management and suppression of existing populations. In Guam the use of virus is aimed to assist eradication and thus the methods of introduction should be modified accordingly.

Virus infection weakens the infected insect and reduces the life span and fecundity of infected insects. It does not immediately kill them. For this reason only males should be released into the field as infected females may still produce some viable eggs and add to the field populations. This will become increasingly important as the population declines to very low levels on the pathway to elimination.

Virus will be introduced into the field populations by release of artificially infected beetles. While there are areas of high population, collection from the field, infection and release are feasible. As the population declines beetles will become more difficult to find in the wild necessitating the use of laboratory reared beetles. A laboratory colony has been started but rearing of scarab beetles through their full life cycle has proven difficult for many species. Collection of advanced 3rd instar larvae, pupae and neonate adults is an alternative and should be used to obtain adult beetles for infection and release in the next phase of the programme. After emergence from the pupa, neonate adults take some time to reach maturity and prepare for flight. This may be as long as 45 days (VanMeer 1987). Feeding can be used as an indicator of maturity. For this reason neonate adults should be maintained in their containers with sphagnum moss with minimal disturbance for the first 2 weeks from eclosion. After 2-3 weeks from eclosion, banana should be presented as a food source and replaced with fresh banana on weekly inspection. Once beetles have commenced feeding they will be ready for infection (double dose over one week) and release (males) or further experimentation (females).

Female beetles can be used for experiments on method of virus infection, strain of virus and impact of virus infection on longevity and fecundity. After infection with virus female beetles should be maintained in damp sphagnum moss and assessed weekly for feeding, weight and survival. Beetles can be removed after one month from infection for assessment of disease (swollen gut), PCR determination of virus and histology. Observations should continue until death (expected after one to two months in virus treated insects).

Male beetles for release should be treated with two doses of virus applied onto sections of banana. After confirmation of feeding on two sections of banana these beetles are assumed to be infected with the virus and can be released in the field.

A managed release programme should be developed. Releases should take place first in areas of high populations (Tumon village, Agana Swamp). Release of a group of male beetles (about 20) is recommended in any area. The beetles should be released in the late afternoon/evening and in small groups across several sites in the release area. Beetles for release should be marked on the elytra and the area should be monitored with pheromone traps. Beetles caught in the traps should be tested by PCR for the presence of virus.

Overall impression

The programme for CRB eradication on Guam is reaching a critical stage. It has been very effective in limiting spread but, on the evidence of pheromone catches, has yet to remove the threat of the beetle. Identification of unmanaged palms and alternative rotting trees as sites of development will enable sanitation efforts to be refocused and provide a further limit to breeding. Elimination of palms which are acting as

breeding sites in the unmanaged areas should reduce emergent beetles which should be quantified in reduced pheromone trap catches.
Successful introduction of the virus will reduce the vigour of the CRB populations and will assist current eradication strategies. Virus should be introduced firstly into areas of high populations where transmission and spread are likely to be rapid. As the overall population declines, managed release may be necessary to assist elimination of the low density populations remaining.

Timetable targets for Phase 2

Dose and release of virus treated beetles. First batch	June 2010
Confirmation of infection in treated female beetles by PCR and histology	August 2010
Determination of impact of virus on beetle longevity and fecundity	Oct 2010
Release of beetles and confirmation of virus spread through PCR	Dec 2010
Review of virus programme	March 2011

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Contract objectives

1. **Fresh samples of tissue culture virus to be supplied by Sean Marshall (Strains X2B, Malaysian B).**

Samples received in Guam 28 May.

Each of the 82 tubes contains 0.5mL (10x 50uL doses) of *Oryctes* virus of the following production lots
-14 tubes of g3 (X2B at 1.8E+07 IU/mL),
-14 tubes of g4 (X2B at 1.36E+06 IU/mL),
-20 tubes of g5 (X2B at 1.31E+06 IU/mL),
-14 tubes of g6 (B at 1.56E+07 IU/mL),
-14 tubes of m1 (B at 5.7E+06 IU/mL),
-6 tubes of m2 (B at 2.28E+07 IU/mL),

2. **Sample of wild type virus from insect guts to be supplied by Shareen Prasad.**

Collection arranged and samples collected in Fiji and sent to NZ. Some problems with transfer. Samples to be assessed and quantified in New Zealand before dispatch to Guam.

3. **Fresh sampling packs will be delivered by Jackson (May 2010).**

Completed. Two sample transfer packs taken to Guam. One used to transport samples collected during the visit. The other left for further samples. More packs with EtOH or FAA tubes to be sent to Guam for sample transfer. (Five packs suggested).

4. **Bioassay plan to be reviewed (Jackson and Moore)**

Key limitation – numbers of healthy beetles

Need for rearing;

1. Full cycle in the lab
2. Field collected and raised through to adults

4.1. Challenge test to ensure uptake and infection by virus in Guam beetles

Key objective is to show that Guam beetles become infected and show symptoms of disease prior to release into the field. Secondarily it will be important to show that mortality, reduction in fecundity and reduction in vigour accompany infection.

Treatment plan
Need for a simple and effective method for dosing and infection to test the virus and to prepare infected beetles for release to spread the virus. Key variables will be dosing method, virus preparation and vigour of beetles.

Beetles selected for testing must be vigorous and capable of flying after infection to distribute the virus. These can be field collected as neonate adults emerging from breeding sites or neonates bred from field collected larvae and pupa or from a continuous insect culture.

Each beetle for testing should be labelled, elytra measured, weighed and fully categorised. Site of origin, stage collected, dates of significant development.

Each beetle should be held individually in a conserve jar (250 ml) with 20 cm of sphagnum moss. The beetles should be tested weekly for feeding with a small section of banana. Feeding beetles should be selected for assay.

Dosing methods

Two dosing methods are available;

Droplet delivery to the mouthparts
Traditional method for virus delivery used by SPC. Virus is mixed with sucrose and dispersant (Tween). Beetles should be kept in dry conditions overnight so that they will readily absorb the droplet. Multiple small droplets will provide greatest chance of success.

Food incorporation assay
Addition to food ensures that the virus is ingested by the beetle. A droplet of virus is applied to the surface of a food source such as banana or sugar cane. Calculation of proportion consumed allows estimation of the dose ingested.

Assessment of treated insects
Virus treated insects should be assessed each week. They should be removed from the container, weighed, vigour assessed (movement, grip) and proportion of food consumed in the week estimated. Treated and untreated control insects should be assessed until most have died.

Experimental design
All experiments should have replicated treatments and controls. Experimental insects should have a specific number and be randomly allocated to treatments. As insects will be available in small batches, experiments may be completed with different batches built up over time.

5. **Adult beetles to be re-dosed with fresh virus supplied by AgResearch and SPC (Jackson and Moore).**

Experiment 1. Infection of adult beetles and preparation for release with cell culture virus from AgResearch (Batch 2).

Beetles from the rearing programme were selected after showing signs of feeding on banana and separated into two groups, male and female. Male beetles will be infected and released into the field. Female beetles will be used for infectivity, mortality and other experiments.

Fresh virus stocks were received from AgResearch and held under refrigeration (4^{o}C). Virus was supplied in sealed tubes containing 0.5 ml. The virus was diluted 1:1 with distilled water to make up a suspension of 1.0 ml.

Beetles collected from the field or raised through the laboratory were held individually in conserve jars in sphagnum moss. Beetle size (elytra measurement), weight, sex and capture and rearing data were recorded. Beetles were provided a piece of ripe banana (approx 5g??) and once feeding had been confirmed the beetles were selected for dosing.

Treatments

1. Droplet (100ul) of diluted virus X2B applied to surface of fresh cut banana
2. Control (100ul clean water)

All male beetles should be treated with virus. For female beetles the treatment to control ratio should be 2:1.

Trial design

Male beetles

- Select after first feeding indicated from laboratory rearing.
- Provide with banana dosed with virus
- Check after 2-3 days to ensure banana has been consumed
- Provide a second banana dosed with virus
- Check again for signs of feeding
- Allocate to release group (should be released in groups of about 20 individuals).

Female beetles

- Select after first feeding indicated from laboratory rearing.
- Allocate randomly to control (1) and treatment (2) groups
- Provide with banana dosed with virus
- Check after 2-3 days to ensure banana has been consumed
- Provide a second banana dosed with virus
- Check again for signs of feeding

- When feeding of the virus impregnated banana has been confirmed place in a long term monitoring group (should aim for 15 control insects and 25 virus treated for the experiment).
- Monitor weekly for survival and weight and provide fresh banana
- At one month from treatment every 3rd insect should be sacrificed and the gut extracted with samples placed in EtOH and FAA (5 control samples and 8 treated samples). The fixed samples should be sent to AgResearch for analysis.
- Continue monitoring the remaining females until most are dead.

Beetles should be weighed and refed with banana weekly. Care should be taken to avoid cross contamination from virus treated to untreated control insects. (Control insects should be examined first in each assessment. The bench should be wiped with an antiviral solution (Virkon) and adults should be examined on fresh clean paper for each group.

Experiment 1. Infection of adult beetles and preparation for release with cell culture virus from AgResearch (Batch 2).

First set of beetles dosed by Trevor Jackson on 3 June 2010.

Infecting males for release

Number	Sex	Feeding date	Treatment	Feeding check 1	Redose	Feeding check 2	Male release date	Release site
869	M	3-Jun	X2B					
870	M	3-Jun	X2B					
871	M	3-Jun	X2B					
873	M	3-Jun	X2B					
876	M	3-Jun	X2B					
877	M	3-Jun	X2B					
879	M	3-Jun	X2B					
884	M	3-Jun	X2B					
411	M	3-Jun	X2B					
926	M	3-Jun	X2B					
928	M	3-Jun	X2B					

Infected females for pathology and efficacy studies

Number	Sex	Feeding date	Treatment	Feeding check 1	Redose	feeding check 2	Feeding	Survival

866	F	3-Jun	X2B					
878	F	3-Jun	X2B					
880	F	3-Jun	Control					
402	F	3-Jun	X2B					
403	F	3-Jun	Control					
404	F	3-Jun	X2B					
407	F	3-Jun	X2B					
409	F	3-Jun	Control					
941	F	3-Jun	X2B					

6. **Treated and field collected beetles will be dissected and guts prepared for analysis (Jackson and Moore).**

a) Samples taken for background virus test (reconfirmation of Prasad data)

Adult beetles were taken from the pheromone catch traps. Location and date were recorded. Insects were dissected and notes made on internal condition and photographs taken. A section of gut (approx 2 cm) was removed, cut in half with one section placed in EtOH and the other in FAA for processing. None of the beetles examined showed any symptoms of virus infection of the gut. Many females had high numbers of eggs present in their oviducts (Up to 29 in one female).

Summary results are provided in Appendix for comparison with molecular and histological data.

7. **Release and sampling plan to be reviewed (Jackson and Moore).**

The release and sampling plan was discussed by Trevor Jackson and Aubrey Moore. The primary decision was only to release infected male beetles. In an eradication programme the release of fecund female beetles, even if infected, could be counter-productive. Releases of male infected beetles should take place in the late afternoon/evening at marked sites with heavy infestations. Released beetles should be marked on their elytra to provide capture release data for population estimates. Releases should be made in groups of 20-30 individuals. Populations on site should be monitored by pheromone trapping.
Once infectivity is confirmed from lab data and transmission is confirmed from trap catches the release programme should be reviewed and plans made to optimise spread of the virus.

8. **PCR assessment to be carried out by SPC (Prasad).**

Twenty samples were collected by Trevor Jackson and Phil Santos for PCR evaluation of background virus from pheromone trap catches. A sampling programme has been established to provide samples from virus treated adult female beetles from the lab trials for confirmation of virus persistence in the gut and samples from the pheromone traps after release to provide confirmation of spread and transmission of the virus. Samples to be sent via AgResearch before sub-sampling and transfer to SPC, Fiji.

9. **Histology assessments to be carried out by AgResearch (Marshall).**

Fourteen samples of CRB adult gut tissue from various sites have been collected for for histology and assessment by Sean Marshall, AgResearch. A sampling programme has been established to provide samples from virus treated adult female beetles from the lab trials for confirmation of infectivity and samples from the pheromone traps after release to provide confirmation of spread and impact of disease.

10. **Data to be reviewed by Jackson, Moore and Campbell.**

Outbreak data, intensity of attack and impact of control measures were reviewed by Trevor Jackson, Aubrey Moore, Russel Campbell, Roland Quituigua, Paul Bassler and other members of the team. The results from pheromone trap catches indicate that spread of the beetle has been slowed, and almost halted, by the intensive sanitation campaign. Data also suggested that CRB behaviour in the hot zones was unusual and led to the important finding that the palm crown was a site of full beetle development, apparently unique to Guam.

Supplementary observations.

Visit to Tumon outbreak site

Rhinoceros beetle was first reported from capture of a beetle adult in Tumon in 2007. A survey revealed that larval populations were present in dead palm stumps and damage could be seen on the emerging palm fronds. The area has been subjected to extensive clean-up of potential breeding material and trapping of adults. Damage however has remained high with most palms in the area showing high levels of attack despite few larvae being encountered in ground surveys and low pheromone trap catches of adult beetles.

The persistent beetle attack in the Tumon area requires examination of the characteristics of the area. Tumon is a beachfront community with entertainment, commercial and residential properties. The area has a scattering of coconut palms along the seafront and throughout the properties. There are some large sections which are unused and overgrown and contain a mixture of vegetation including palms.

The overgrown areas have been cleared of dead palm trunks and are regularly inspected by the beetle eradication team with few beetles encountered in the ground studies. High amounts of residual organic matter in the crown of the damaged palms prompted the team to check the possibility that the beetle life cycle was being completed in the organic matter in the crown of the plant. The beetle team visited the site and cut down three suspect palms. Eggs and larvae of rhinoceros beetle, up to the pre-pupal stage, were found in two of the three palms indicating that the full life cycle can take place in the palm crown and emergent adults from these palms are probably responsible for the damage seen on the site. Evidence of rhinoceros beetle larvae and development was also found in the old and decaying flame tree stems found on the site. These should also be treated as a potential source of damaging beetles. Removal of unmanaged palms containing high levels of organic matter in the crown is recommended.

Beetle rearing facility

Well set up
Divided into entry space, rearing and assessment areas.
Double door and locking systems good for security
Materials leaving the unit should be sterilised by autoclave or chemical means.
Basic facility, individual rearing
Separation of field collected and colony reared individuals
Small larvae 2nd and 3rd instar should be reared collectively in containers
Moisture level of the breeding material is very important. The rearing material, dried steer manure will absorb a high percentage of moisture. Satisfactory water content of the medium should be determined through rate of growth assessment and containers filled to a fixed weight. Containers should be checked weekly and water added to the target weight.
Third instar larvae should be reared in individual jars. The preserve jars are very good for this. Feed material should be added and replaced weekly.

1. Use a standard rearing material
2. Prepare to a fixed water content suitable for rearing. Place in sealed containers ready to use.
3. Add feeding material to small larvae rearing containers to a set weight. Mark on the side of the container. Check wt weekly and add water to the set weight.

Appendix 1

Introduction of *Oryctes* virus for control of rhinoceros beetle, Guam

Summary of activities and progress to date

2 May 2010

Background

Rhinoceros beetle (*Oryctes rhinoceros*) was discovered as an invasive insect pest in Guam in 2007. The population has persisted despite attempts at eradication through trapping and clearing of breeding sites. In 2009 it was proposed to introduce *Oryctes* virus as a biological control agent to contribute to the eradication of the pest. A programme was developed with AgResearch, Sada Nand Lal and SPC for introduction and management of the virus and a proposal approved by USDA for funding.

Phase 1. Progress to date

1. Virus supply (Sean Marshall)

A shipment of *Oryctes* virus (4 vials of 0.5 mL) was supplied by Sean Marshall, AgResearch for testing. Vials (2 mL plastic screw cap tubes with silicon seal) were sent directly to USDA APHIS PPQ via courier (see Appendix 1 for letters included with shipment). Shipment was sent from AgR (Lincoln, New Zealand) on 14/09/2009, and arrived at USDA APHIS PPQ (Guam) on 21/09/2009.

The *Oryctes* virus strain sent was the biocontrol standard X2B, produced using insect cell culture line DSIR-HA1179 (production lot X2B-b 09.2.2). Virus concentration was estimated by titration just prior to sending on 11/09/2009 and indicated a titre 1.6×10^6 IU/mL (based on $TCID_{50}$ assay using the cell line). The virus stock remaining at AgResearch from the batch made for the Guam shipment was retitred again on 04/05/2010 (5.5×10^6 IU/mL). The values are within the calculated 95% confidence interval obtained for the 11/09/2009 sample.

2. Dosing and release (Sada Lal and Aubrey Moore)

Sada Nand Lal visted Guam in September 2009. Pre-release samples were collected by Univ Guam and dissected on 22-09-09 (Prasad report). Ten beetles were dissected and all had typical healthy darkened guts. Samples were prepared for PCR and histological analysis.

3. PCR testing (Shareen Prasad)

PCR testing of the ten pre-release samples was carried out by Shareen (Prasad report 2009) and all samples were negative for the presence of virus.

4. Follow-up (Aubrey Moore)

Bioassay, release and monitoring
Twenty-four beetles were dosed and released.
Most beetles show no sign of infection (swollen gut)
One beetle (Ringo) dosed with virus is still alive and apparently healthy several months after treatment.
Samples taken for PCR detection were withheld by the Fiji customs and destroyed.

Documents;

Shareen Prasad. Agricultural Biotechnology, Guam Technical Report, Year 2009.

Phase 2

Concern has been raised about uptake of the virus and the following work plan is proposed for Phase 2. Trevor Jackson will visit from (30 May to June 5, 2010).

1. Fresh samples of tissue culture virus to be supplied by Sean Marshall (Strains X2B, Malaysian B).
2. Sample of wild type virus from insect guts to be supplied by Shareen Prasad.
3. Fresh sampling packs will be delivered by Jackson (May 2010).
4. Bioassay plan to be reviewed (Jackson and Moore)
5. Adult beetles to be re-dosed with fresh virus supplied by AgResearch and SPC (Jackson and Moore).
6. Treated and field collected beetles will be dissected and guts prepared for analysis (Jackson and Moore).
7. Release and sampling plan to be reviewed (Jackson and Moore).
8. PCR assessment to be carried out by SPC (Prasad).
9. Histology assessments to be carried out by AgResearch (Marshall).
10. Data to be reviewed by Jackson, Moore and Campbell.