

Additional Documentation in Support of Permit Application P526-190319-001

Prepared by Aubrey Moore PhD, Entomologist, University of Guam
June 2, 2019

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1. Background and Justification

On March 19, 2019 I requested a permit to import live coconut rhinoceros beetles (CRB), *Oryctes rhinoceros*, to Guam (Application number P526-190319-001) to replace permit P526P-11-01844 for this purpose (Appendix A), which I accidentally allowed to lapse into oblivion after only one shipment.

On May 10, 2019 I was sent automated email from the USDA-APHIS permit office requesting additional information in the form of an Applicant Inspection Questionnaire. This document is a response to that request.

I request permission to import live coconut rhinoceros beetles, *Oryctes rhinoceros*, into Guam. These beetles will be used in USDA-APHIS funded laboratory research

aimed at developing biological control for this species. No imported beetles or their progeny will be released on Guam.

Massive mortality of coconut and other palms is occurring on Guam and several other Pacific islands as the result of uncontrolled outbreaks of coconut rhinoceros beetle biotype G (CRB-G). Pacific island invasions of CRB where previously controlled by a microbial biological control agent, *Oryctes rhinoceros* nudivirus (OrNV). Unfortunately, CRB-G appears to be resistant to all currently available isolates of OrNV. My laboratory is funded by USDA-APHIS Farm Bill grants to find new isolates of OrNV which can be used for effective biological control of CRB-G. We are importing OrNV samples under Import Permit P526P-17-03146 and are evaluating these as potential biological control agents in laboratory bioassays.

We have established a laboratory colony of CRB-G to supply insects for bioassays. But we also need to establish a laboratory colony of virus-susceptible beetles for our tests. We request permission to import beetles from foreign sources because CRB-G is the only biotype known to exist on Guam.

2. Quarantine Facility

The University of Guam operates a USDA Biological Control Facility in House 35, Dean's Circle, University of Guam Campus, Mangilao. I am not intending to use this facility for my project. Instead, I request permission to contain beetles in room 319, Agriculture and Life Sciences Bldg, University of Guam Campus, Mangilao because:

- The House 35 site does not have environmental chambers required for establishment and containment of a laboratory colony and for containment of insects during bioassays.
- If beetles were kept in the House 35 facility, this would require carrying beetles outdoors between buildings in order to perform bioassays and to make daily observations.

3. Standard Operating Procedures

3.1. Compliance

All conditions specified in permit will be met.

3.2. Transportation

We have developed a highly secure container for shipment of CRB (Appendix B). This container will be used for all shipments.

All packing material and insect bedding material (peat moss) will be autoclaved prior to final disposal.

3.3. Rearing conditions

Imported CRB will be reared individually in Mason jars enclosed by metal caps Fig. 1.



Figure 1: CRB are reared individually, in Mason jars with metal caps.

Mason jars will be in environmental cabinets in a locked room with sealed windows (Room 319, Agricultural and Life Sciences Building, University of Guam, Mangilao, Guam)Fig. 2.

3.4. Record keeping

Detailed records for each individual beetle will be stored in an on existing on-line laboratory information management system (LIMS). These data will be made



Figure 2: Environmental cabinets in Room 319, Agricultural and Life Sciences Building, University of Guam, Mangilao, Guam.

available to USDA-APHIS.

4. Note on Risk

CRB are very large scarab beetles and are more easily contained than smaller insects which can pass through small openings such gaps around doors. Our beetles are kept individually inside sealed Mason jars inside sealed environmental chambers inside a locked room. If the suggested containment protocol is followed, it is very unlikely that any beetles will escape into the Guam environment. However even if foreign CRB escaped and started to breed, this would not measurably increase damage beyond what is already present on Guam (Fig. 3).



Figure 3: Damage

**A. Expired Permit to Import Coconut Rhinoceros
Beetles P526P-11-01844**

Please see next page.



United States Department of Agriculture
Animal and Plant Health Inspection Service
4700 River Road
Riverdale, MD 20737

Permit to Move Live Plant Pests, Noxious Weeds, and Soil
Importation
Regulated by 7 CFR 330

This permit was generated electronically via the ePermits system

PERMITTEE NAME:	Dr. Aubrey Moore	PERMIT NUMBER:	P526P-11-01844
ORGANIZATION:	University of Guam	APPLICATION NUMBER:	P526-131217-017
ADDRESS:	College of Natural and Applied Science University of Guam Mangilao, GU 96923	FACILITY NUMBER:	N/A
MAILING ADDRESS:	College of Natural and Applied Science University of Gum Mangilao, GU 96923	HAND CARRY:	Yes
PHONE:	(671) 735-2086	DATE ISSUED:	12/19/2013
FAX:		EXPIRES:	05/10/2014
DESTINATION:	College of Natural and Applied Science, University of Guam, Mangilao, GU 96923 cafe		
DESIGNATED PORTS:	GU, Agana		

Under the conditions specified, this permit authorizes the following:

Article Category: Invertebrate Pests - Insects

<u>Regulated Article</u>	<u>Life Stage(s)</u>	<u>Intended Use</u>	<u>Shipment Origins</u>	<u>Originally Collected</u>	<u>Culture Designation</u>
Oryctes rhinoceros	Any	Research - Lab	China, Palau, Philippines, Fiji, Samoa, Tonga, Papua New Guinea, Malaysia, Indonesia, Asia	Originally Collected from Foreign and Domestic Locations	

SPECIAL INSTRUCTIONS TO INSPECTORS

See permit conditions below

INSTRUCTIONS TO DHS CBP INSPECTORS:

1. Confirm that the carrier of the shipment imported under this USDA PPQ 526 permit is commercially bonded. For all hand carry events, go to step 6.
2. Confirm that the imported shipment has a valid USDA PPQ Form 599 Red/White label attached to the exterior for routing to a USDA APHIS PPQ Inspection Station. (A valid label will have the permit number, expiration date, label number, and address of a USDA APHIS PPQ Plant Inspection Station)
3. Validate the permit in ePermits using the CBP search feature.
4. If a valid PPQ Form 599 Red/White label is not attached to the exterior of the package or the label has been covered or is otherwise not legible, then forward to the nearest USDA APHIS PPQ Plant Inspection Station.
5. If the address on the airway bill does not match the address on the PPQ Form 599 Red/White label then forward the package to the nearest USDA APHIS PPQ Plant Inspection Station. (All costs associated with rerouting misaddressed

Permit Number P526P-11-01844

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING
PPQ HEADQUARTER OFFICIAL VIA EPERMITS.

Colin Stewart

DATE

12/19/2013

WARNING: Any alteration, forgery or unauthorized use of this Federal Form is subject to civil penalties of up to \$250,000 (7 U.S.C.s 7734(b)) or punishable by a fine of not more than \$10,000, or imprisonment of not more than 5 years, or both (18 U.S.C.s 1001)



packages will be assumed by the permit holder.)

6. For all hand carry events, the port of entry must have received prior notice by e-mail, fax, or telephone of the arrival of the passenger including date of arrival and flight information from Headquarters, OFO, and APL. This notice communicates specific instructions to the port of entry.
7. For all hand carry events, a valid red and white PPQ Form 599 Red/White label specifying hand carry must be presented. A single PPQ Form 599 Red/White label specifying hand carry may be used for multiple specimens covered under one permit at border crossings.
8. If the permitted shipment arrives by hand carrying, in personal luggage, or by personal automobile without a hand carry authorization as specified in both the permit and the PPQ Form 599 Red/White label, seize the shipment and forward it to the nearest USDA APHIS PPQ Plant Inspection Station.
9. For questions or concerns, contact the USDA APHIS PPQ Permit Unit in Riverdale, MD, at 301 734-6343/4404.

INSTRUCTIONS TO USDA PPQ INSPECTORS AT PLANT INSPECTION STATIONS:

If any of the following are not met, DO NOT CLEAR THE SHIPMENT. Contact the headquarters Permit Unit at 301 734-6343/4404 to determine subsequent action.

1. Confirm that the package contents are listed on the permit.
2. Confirm that the shipment has the original PPQ Form 599 Red/White label with the permit number and label number visible on the exterior of the package.
3. If the PPQ Form 599 Red/White label is absent (the label may have become detached during shipping) altered or otherwise illegible, then proceed to step 14 below.
4. Confirm that the PPQ Form 599 Red/White label is addressed to your Plant Inspection Station Address.
5. Confirm that the delivery address on the package matches the address on the PPQ Form 599 Red/White label.
6. Confirm that the permit number on PPQ Form 599 Red/White label matches the actual permit number.
7. If the PPQ Form 599 Red/White label has a barcode, scan the barcode, or enter the permit number and label number into ePermits and log the required shipment information. If the PPQ Form 599 Red/White label does not have a barcode, validate the permit using the ePermit search feature.
8. Confirm that the permit has not expired.
9. Confirm that the final destination of the package is to the same individual and address that appears on the permit.
10. Confirm that the origin of the shipment is authorized on the permit.
11. Use a biosafety cabinet to inspect shipments of microorganisms.
12. Confirm that the package is adequate to prevent escape of the enclosed organisms.
13. If all permit conditions are met, release the package for subsequent movement to the permit holder's address.
14. Do not open the package outside of a biosafety cabinet.
 - If your facility does not have a biosafety cabinet and the package is secure, re-export the package.
 - If your facility does not have a biosafety cabinet and the package is not secure, destroy the package.
 - If your facility has a biosafety cabinet, examine the contents for a PPQ permit or enclosed supplemental label information.
 - If the package contains a PPQ permit or supplemental label information, proceed to step 7 above. (Supplemental label information includes the permittee's letterhead stationary with their name, permit number and label number. This information enables you to use the ePermit barcoding feature if the missing label was barcoded, or validate the permit in ePermits using the search feature if the label was not barcoded.)
 - If the package does not contain a PPQ permit or supplemental label information and the package is secure, re-export the shipment.
 - If the package does not contain a PPQ permit or supplemental label information and the package is not secure, destroy the shipment.

PERMIT CONDITIONS

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This permit authorizes the importation of all stages of Oryctes rhinoceros from the listed regions by Aubrey Moore, College of Natural and Applied Science, University of Guam, Mangilao, GU 96923, for laboratory research in USDA APHIS Inspected Facility # 2348.

The permit holder is authorized to hand carry these insects if all permit conditions are met.

1. Regulated organisms must be maintained only in the laboratory area at the work address indicated on this permit. Regulated organisms must be removed from the laboratory or other designated area only with prior authorization from APHIS. Only the evaluated area is authorized for containment. Other rooms require specific approval.
2. Standard Operating Procedures (SOPs) must be current and on file with the Pest Permit Evaluation Unit, 4700 River Road Unit 133, Riverdale, MD 20737 Facsimile 301-734-8700, (866) 524-5421; email pest.permits@aphis.usda.gov.
3. All persons working with these regulated organisms must be trained on the importance of approved containment practices.
4. This authorization is not valid for field research, greenhouse work, or release into the environment.
5. Regulated organisms must be destroyed as soon as they are no longer needed but no later than the expiration date of this permit. Apply for a new permit at least 3 months before the expiration date of this permit.
6. All waste materials from these cultures and any associated shipping materials must be disposed of properly. All waste materials must be double bagged and prepared by one of the following methods prior to disposal: 1) frozen at -20F for 72 hours (note this is colder than most refrigerator freezers), 2) autoclave, 3) incinerated, before entering the municipal waste stream.

If an autoclave is used then waste must be autoclaved at at least 121 degrees Centigrade (250 degrees Fahrenheit) for a minimum of 30 minutes.

Autoclave tape or other indicators must be placed on each bag or sharps container prior to treatment. The autoclave tape or other indicator on each container must be checked to verify color change before disposal.

The autoclave log must be completed by each user for each autoclave cycle. All parameters must be noted as listed on the log for each autoclave load.

If the autoclave does not attain the minimum time and/or temperature or the autoclave tape does not change color, annotation must be made in the comment section of the autoclave log. The load must then be re-autoclaved after placing new tape on the material. If minimum time and temperature is not attained on the second cycle, users must contact the person responsible for maintaining the unit to initiate repairs. Waste should then be treated at the alternate autoclave facility.

Thermometers on the autoclave should be calibrated annually, and a written record must be maintained. This should be done by an authorized autoclave service company during routine servicing.

Every 6 months, you should use a commercially available test indicator kit that uses bacterial spores Bacillus stearothermophilus that are rendered unviable at 250 degrees F or 121 degrees C. For the test, ampoules of B. stearothermophilus are autoclaved along with a load of waste. Upon completion of the cycle, the ampoules are incubated for 48 hours and then observed for any sign of growth, which would indicate that the autoclave is not sterilizing properly. If any growth is observed, have autoclaved serviced and retest.

7. All living organisms isolated from these regulated organisms must be maintained in the laboratory only. No field release is authorized under this permit.
8. All persons working with these organisms must be informed of these permit conditions. Anyone working with these insects must agree to and sign/ initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/ APHIS but must be readily accessible in the event of an inspection and presented upon request.
9. The permittee is responsible for reviewing the US State Department's current restrictions on exchanges with certain countries, such as (but not limited to) Burma (Myanmar), Cuba, Liberia, North Korea, Iran, Iraq and Libya, before requesting plant material. The permittee should check the State Department's web site and contact that Department directly if uncertain about current sanctions.

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10. There is to be no further distribution of these organisms without prior approval from State and Federal regulatory officials. Anyone requesting to receive these organisms from the permittee must possess a valid PPQ 526 permit to receive them.
11. Modifications to the containment of these organisms must be approved prior to making changes. Please contact the Permit Unit, 4700 River Rd., Unit 133, Riverdale, MD 20737, Facsimile 301-734-8700, (866) 524-5421; email: pest.permits@aphis.usda.gov.
12. All containers for transport (hand carry if applicable, or shipping) will be at least double-packed and adequately packaged to prevent the escape/leakage of the enclosed organisms.
13. This permit only covers the listed arthropods. Work with plant diseases that these arthropods may transmit originating from outside of the destination state requires a separate permit.
14. This permit is issued only for the named permit holder at the address(s) identified on this permit. This permit cannot be transferred or assigned nor does it fulfill or modify the requirements of any other federal or state regulatory authority (such as the U.S. Environmental Protection Agency, the U.S. Fish and Wildlife Service, the U.S. Food and Drug Administration, the Centers for Disease Control and Prevention, the Animal Health Protection Act- 7U.S.C. 8301, or your State's Department of Agriculture).
15. The permit holder verifies United States residency by initialing and accepting these permit conditions. If you are not a United States resident, it is unlawful for you to initial or accept these permit conditions because a USDA 526 Plant Pest Permit can only be issued to United States residents.
16. The permit holder is solely responsible for ensuring compliance with all statutory requirements and specifically listed permit conditions. Failure to comply with the terms and conditions of this permit is cause for the following: (a) cancellation of this permit, (b) cancellation of other permits issued to the permit holder, (c) seizure and/or destruction of regulated organisms, (d) denial of future permit applications by this permit holder, (e) liability for civil penalties, and (f) criminal prosecution under provisions in the Plant Protection Act.
17. Any alteration, forgery, unauthorized use of this permit and/or associated Federal Forms are subject to civil and criminal penalties including fines and imprisonment.
18. Importation, interstate movement, possession, and use of strains of genetically engineered regulated organisms (created by use of recombinant DNA technology) are not authorized under this permit.
19. This permit does not authorize movement or use of plant pathogens listed in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. If any organism listed as a Select Agent is identified from materials associated with this research, the permit holder is required to notify APHIS, Agricultural Select Agent Program (ASAP) immediately by phone at 301-734-5960, and within seven (7) days submit APHIS/CDC Form 4 (Report of Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory) to APHIS, ASAP; 4700 River Rd, Unit 2, Riverdale, MD 20737 (see instructions at: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml). Failure to comply with this requirement is a violation of the Agricultural Bioterrorism Protection Act of 2002.
20. If organisms that are not authorized in this permit are received, the permit holder must take all prudent measures to contain the organism(s) and notify the PPQ permit unit by contacting a compliance officer immediately (that is, within one business day) by calling 866-524-5421 or by e-mail to pest.permits@aphis.usda.gov. The permit holder must immediately notify the permit unit of the destruction of regulated organisms received under this permit, as above. Similarly, the permit holder must immediately notify the permit unit if facilities are destroyed or decommissioned for any reason.
21. The permit holder must maintain a valid permit so long as the regulated organisms are alive and in your possession. The permit holder must safeguard and dispose of the regulated organisms during the term of this permit. This permit cannot be extended or renewed. A new permit is required for uninterrupted authorization/use of regulated organisms after this permit expires.
22. The permit holder must take all necessary precautions to prevent the escape of plant pests. In the event of an escape, the permit holder must notify the permit unit within one business day by calling 866-524-5421 or by e-mail to pest.permits@aphis.usda.gov. The permit holder must adequately mitigate any and all environmental impacts resulting from unauthorized release of organisms received under this permit.

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23. Without prior notice and during reasonable hours, authorized PPQ and/or State regulatory officials shall be allowed to inspect the conditions associated with the regulated organisms authorized under this permit.
24. The permit holder must maintain an official permanent work assignment at the address identified on this permit. If the permit holder ceases assignment/affiliation at the address identified on this permit, or personnel circumstances change in any way, then a compliance officer must be notified at the PPQ permit unit immediately (that is, within one business day) by either (a) email to pest.permits@aphis.usda.gov, (b) fax to 301-734-4300 or 8700, or (c) conventional mail to USDA PPQ Permit Unit, 4700 River Road, Riverdale, MD 20737. The permit holder must destroy all regulated organisms prior to departure unless the permit holder either (a) requests cancellation of this permit and complies with all permit-specific termination conditions, (b) applies for and receives a permit to move the organisms to a new facility, or (c) relinquishes control of the regulated organisms to a qualified individual who obtained a permit for the continued use of these regulated organisms prior to this permit holder's departure.
25. Upon issuance of this permit (i.e., a signed PPQ 526), you will need to request the PPQ Form 599 Red/White labels. If you applied online using ePermits, you may request the labels using the My Shipments/Labels feature. Otherwise, send your request to redandwhitelabelrequest@aphis.usda.gov. Specify the approved port as listed on the permit and the total number of labels needed in a multiple of eight. You may request additional labels the same way. We will send you the labels using a bonded carrier.

A label must be attached to the exterior of each package being imported under this permit. The labels have detailed instructions for use on the reverse side. You are responsible for instructing your shipper to carefully follow these instructions. You are responsible for each import shipping label issued under this permit.

Enclose the following supplemental information in each shipment:

- Permittee Name
- Permit number
- Label number

Underlying packaging/wrapping must carry the address, billing, and any other information required to direct the shipment to its final destination (i.e., the permit holder's address; N.B., USDA APHIS does not defray any additional shipping costs incurred for transiting the shipment through an inspection station as the initial US destination).

26. EACH HAND CARRYING EVENT MUST BE PREAUTHORIZED BY USDA APHIS BY PROVIDING THE INFORMATION REQUIRED BELOW.

The following conditions apply to all hand carry authorizations:

1. At least TWENTY days prior to each hand carry incident, the permit holder or designee must notify the PPQ Permit Compliance Officer by email (redandwhitelabelrequest@aphis.usda.gov) or telephone (866-524-5421) to provide specific information on the hand carrier's identity, the anticipated first port of arrival into the United States, the actual date of arrival, the time, and, if travel is by airline, the flight number. The Compliance Officer will notify Customs and Border Protection (CBP) Agriculture Specialist at the port of entry to document and facilitate the entry of the organisms.
2. The hand carrier must indicate that living organisms are being imported under a USDA permit on the Customs Declaration form if such form is required at the port of entry.
3. At the port of entry, individuals carrying permitted organism must also present to CBP officers the following articles: U.S. Passport, U.S. Resident Alien Card, or Visa and a valid hand carry PPQ Form 599 Red/White label corresponding to the permit.
4. Inspection by CBP Officers must confirm that all hand carried articles are securely packaged as per the permit conditions. In the event that a problem is detected, the CBP officer may seize the package and require its movement to the nearest PPQ Inspection Station for processing, clearance or destruction. The permit holder will be responsible for all costs incidental to such forwarding.
5. After CBP confirmation and clearance through the first port of entry into the United States, hand carried organisms must be transported directly to the containment facility authorized in the permit.
6. Upon arrival at the facility, the PPQ Compliance Officer must be notified within 24 hours that the organisms arrived. Notification may be by fax (301-734-5392) or email (redandwhitelabelrequest@aphis.usda.gov). Notification must be

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by an independent third party (e.g. containment facility director, departmental chair, campus biosafety officer, etc.). The notification must include the permit number, label number, date of arrival, the specific organisms that were imported, their origin, and quantity. Failure to notify the PPQ compliance officer may result in loss of hand carry privileges. A PPQ inspector may also visit the facility to confirm the arrival of the package and its contents.

7. ONLY PERSON(S) WHOSE NAME(S) IS/ARE LISTED IN THE ISSUED PERMIT IS/ARE AUTHORIZED TO HAND CARRY.

8. You will receive NEW PPQ Form 599 Red/White labels for each hand carry event once you have submitted the required information. You can NOT use the red and white labels described above that are prepared for BONDED CARRIERS. If you use the PPQ Form 599 Red/White labels for bonded carrier while attempting to hand carry, the package will be seized by the Department of Homeland Security and destroyed.

END OF PERMIT CONDITIONS

Permit Number P526P-11-01844	
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B. Secure CRB Shipping Container

Please see next page.

Guam Coconut Rhinoceros Beetle Project

Technical Report CRB-2017-05-27



Container for Secure Shipment of Live Coconut Rhinoceros Beetle Adults

Prepared by
Aubrey Moore and Roland Quitugua
University of Guam Cooperative Extension Service

Updated June 1, 2017

A few years ago, we were asked to provide live CRB adults to be used in experiments conducted in the laboratory of Dr. Eric Jang at the USDA-ARS Pacific Basin Research Center. Shipments were made under conditions specified by a USDA-APHIS 526 permit issued to Dr. Jang. The first batches of beetles were shipped in Mason jars packed in the cardboard box in which they were purchased. On one occasion, a jar was broken during shipment. Fortunately, the beetle did not escape. This incident prompted us to design a more secure shipping container as described here. CRB adults have been shipped in this container many times without incident.

Notes

1. A small hole (1/32") is drilled at the center of each end cap to provide gas exchange.
2. Prior to shipment, a single beetle is placed in each nipple filled with moist peat moss.

Table 1: Parts list.

Item	Quantity
Pelican case; Model 1520	1
nipples; PVC schedule 80; 3" long; 2" internal diam.	12
end caps to fit above	24
High density Styrofoam	1 sheet
peat moss	1 bag



Figure 1: Shipping case.



Figure 2: Nipple and end caps.