The Dean's 2016 High-Impact Project Pool Competition

Directions/Coversheet

Proposal application due at the Dean' Office by: NOON on Tuesday, March 1, 2016.

Amount of funds requested $39,911_______ (no more than $40,000)

Title of project Coconut rhino beetle as a transmission vector of Tinangaja disease

Issue as described in the Nov 4, 2015 priority listening session:

Agriculture & Gardening: Q1-1 Prevention, Control and Dissemination of invasive species

Team members

Name	*Role/task in the project*
Aubrey Moore___________	_Principal Investigator___________________________
Andrea L. Blas__________	_Collaborator, Project Researcher________________
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Summarize the compelling case for this project in 300 words maximum. (Succinctly state the problem, the magnitude/cost/size of the problem, what issue will continue without your project's intervention, your solution, and how you plan to measure impact/uptake.)

The coconut rhinoceros beetle (CRB), as an invasive species first detected in Guam in 2007, has already made a significant impact on the local environment and economy, with major impact on one of the most important local cultural icons, the coconut palm. Since 2007, CRB has spread throughout the island and approximately 50% of coconut palms in the Tumon Bay area show signs of recent CRB feeding damage (Moore et al., 2015). Several species of palm are damaged by CRB when adult beetles bore into the crowns of palms to feed on sap. Since CRB feed several times, boring into crowns of different palms during their adult stage, it has been suggested that this species may be an efficient vector for transmission of coconut palm viroids. Tinangaja disease, a lethal but slow-acting disease caused by *Coconut tinangaja viroid* (CTiVd), was reported to affect approximately one third (30.4%) of coconut palms in Guam in 1997 (Wall and Wiecko, 1997). Currently, CTiVd is known to exist only in Guam. We propose to (a) confirm the status of CRB as transmission vectors of CTiVd by performing a field survey to determine the correlation between CRB damage and tinangaja incidence and (b) to determine the type of viroid transmission (e.g. transient, persistent) by examining how long CTiVd can be detected within CRB. Our results will be used to revise management recommendations for controlling CRB and Tinangaja.

Application body (4 pages maximum, any way you want to use the space)

Title of project: Coconut rhino beetle as a transmission vector of Tinangaja disease

Issue as described in the Nov 4, 2015 priority listening session:

Agriculture & Gardening: Q1-1 Prevention, Control and Dissemination of invasive species

Problem Statement

The coconut rhinoceros beetle (CRB), as an invasive species first detected in Guam in 2007, has already made a significant impact on the local environment and economy, with major impact on one of the most important local cultural icons, the coconut palm. Since 2007, CRB has spread throughout the island and approximately 50% of coconut palms in the Tumon Bay area show signs of recent CRB feeding damage (Moore et al., 2015). Several species of palm are damaged by CRB when adult beetles bore into the crowns of palms to feed on sap. Since CRB feed several times, boring into crowns of different palms during their adult stage, it has been suggested that this species may be an efficient vector for transmission of coconut palm viroids. Tinangaja disease, a lethal but slow-acting disease caused by *Coconut tinangaja viroid* (CTiVd), was reported to affect approximately one third (30.4%) of coconut palms in Guam in 1997 (Wall and Wiecko, 1997). Currently, CTiVd is known to exist only in Guam. We propose to (a) confirm the status of CRB as transmission vectors of CTiVd by performing a field survey to determine the correlation between CRB damage and tinangaja incidence and (b) to determine the type of viroid transmission (e.g. transient, persistent) by examining how long CTiVd can be detected within CRB. Our results will be used to revise management recommendations for controlling CRB and Tinangaja.

During the feeding stage of adult CRBs, the adults pass through several episodes of flight/feeding to other coconut palms over a period of approximately 120 days. Movement of CRB during this phase is such that approximately 50% of coconut palms in the Tumon Bay area showed signs of recent CRB feeding damage (Moore et al., 2015). This feeding behavior and the dispersal of CRB is highly significant in its potential to spread Tinangaja disease.

Tinangaja is a lethal but slow-acting (taking up to 15 years from infection to palm death) disease of coconut palms that is currently found only on Guam. Tinangaja primarily affects mature, nut-producing coconut palms. The traditional, long-lived coconut variety grown in Guam is most likely to be infected after reaching flowering stage at 25-30 or more years old. During the first stage of Tinangaja, coconut palms show no symptoms and this stage may last several years. In the second stage of Tinangaja typically lasting 5 or more years, the most distinctive visual symptoms emerge: reduced size and deformation of nuts by equatorial scarification leading eventually to mummified nuts with no kernel. The third and final stage prior to palm death is characterized by complete lack of nut production and inflorescences, if present, are sterile (Boccardo et al., 1981; Hanold and Randles, 1991; Hodgson et al., 1998; Wall and Wiecko, 1997).

Transmission methods of CTiVd and the related *Coconut cadang-cadang viroid* (CCCVd) have not been resolved (Frison and Putter, 1993; Sullivan et al., 2012). Cadang-cadang disease of coconut (caused by CCCVd) shows nearly identical symptoms to Tinangaja disease and the two can be reliably distinguished only using molecular diagnostic tests, especially during the early stages of disease. CCCVd is transmissible at low rates by both pollen and seed though viability of resulting seeds is reduced (Pacumbaba et al., 1994). Mechanical transmission through use of non-sanitized blades is considered the most likely mode of transmission but infected pollen carried by wind or insects is also possible. Coleopteran insects, a group that includes CRB, are the suspected insect vectors but this has not been verified (Hanold and Randles, 1991; Hinckley, 1973; Pacumbaba et al., 1994; Sullivan et al., 2012). With recent incursions of CRB on other islands, it is crucial to establish whether CRB are in fact transmission vectors of CTiVd so that containment and monitoring recommendations and programs for CRB and Tinangaja disease may be established.

Objectives

The objectives of this project are to (1) clarify status of CRB as a transmission vector of CTiVd, (2) determine persistence time of CTiVd within CRB and felled coconut palms and (3) revise recommendations for both CRB and Tinangaja disease management.

Methology/Research Approach

To clarify the status of CRB as a transmission vector of CTiVd, we propose a series of short studies using field-collected CRB adults and larvae from CTiVd-infected coconut palms. Coconut palms will be surveyed for CTiVd infection using a commercial CTiVd detection kit (Norgen Biotek Corp., Thorold, Ontario, Canada). Stage of tinan~~nini~~gaja infection, rated according to the scale devised by Wall and Wiecko (1997), and incidence of damage by CRB will be recorded. If CRB is a vector of CtiVd, we expect to see a significantly higher infection rate in coconut palms attacked by CRB.

Field-collected adults extracted from feeding galleries in CTiVd-positive palms will be used for RNA isolation and molecular diagnostic testing to confirm the presence/absence of the viroid as either a surface residue or internal microbiota. Once established, persistence of the viroid with the CRB host will be examined at fixed time points post-collection. We propose also to determine whether (a) CTiVd can be passed from [field-collected] parent to [lab-reared] progeny and (b) whether persistence continues through metamorphosis from [field-collected] larvae to [lab-reared] adult. Additionally, we propose to determine persistence of the viroid within the coconut palm host at fixed time points post-felling in the detached stem and rooted-stump. With data from the above studies we will revise, as needed, current management recommendations for controlling CRB and Tinangaja disease.

Potential Impacts and Expected Outcomes

The major expected outcome is confirmation of CRB as an insect transmission vector for CTiVd. Research results will be submitted for peer-review in a publication such as *Plant Disease.* We expect to determine how long the viroid can persist away from a coconut palm host and thereby provide the basis for further studies to determine the circulation or propagation of the viroid in an insect vector.

Plant viroids are extremely small, ranging from 246 to approximately 400 nucleotides in length (by comparison, *E.coli* has a genome size of 4.6 Million base pairs), and rely entirely on the cellular machinery of a host for replication and transmission from host-to-host. CTiVd is unusual among plant viroids as it is one of only two known viroids to infect a monocot and the next closest related species is CCCVd which shares just ~64% sequence similarity with CTiVd (Keese et al., 1987). What is so different about CTiVd that despite the variability in efficacy in Plant Quarantine programs over the past 100 years, Guam is still the only known site of Tinangaja disease, natural infection or otherwise? With the introduction of new potential insect vectors like CRB, how will this affect the dispersal of Tinangaja to other islands? The potential impact of this study is in demonstration that WPTRC can provide a unique experimental organism (i.e. CTiVd) to establish research collaborations to study the evolution and human-mediated and/or insect-vectored transmission of plant viroids.

For Extension and Outreach, the major expected outcome is revision of current management recommendations regarding Tinangaja disease especially in regards to CRB infestations. Outreach products will include updated print and online publications (on the CNAS and UOG websites) for Tinangaja and CRB. Poster presentations of the research and management recommendations will be targeted for local science-related symposia such as Science Sunday and the CNAS Student Research Symposium as well as presentation at a professional conference such as the American Phytopathology Society Annual Meeting. The major potential impact will be reporting and incorporation of research results and management recommendations for Plant Protection and Quarantine programs and educational workshops such as the 16th Annual Quarantine Training Workshop sponsored by USDA-APHIS, Government of Guam's Department of Agriculture and the Secretariat of the Pacific Community and hosted at UOG during Spring Break 2017.

Task implementation timeline

List the tasks in the left and then put an “X” in the months where the work will be taking place.

Task	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Acquisition of test kits	X	X	X									
ID of CTiVd-infected palms			X									
Persistence in felled palms												
CTiVd testing of palms			X	X								
Optimize CTiVd testing with CRB-G			X	X								
Detection in CRB-G host												
Field collection of CRB-G				X								
CTiVd testing of CRB-G				X								
Persistence in CRB-G host												
Field collection of CRB-G					X							
Lab rear collected CRB-G					X	X	X					
CTiVd testing of CRB-G						X	X					
Revise management recommendations								X				
Prepare factsheets, posters and manuscript									X	X	X	X
Final report												X

Budget (1 page)

Fill out this table with details within the brackets []. For supplies, break them down rather than just saying, $500 for supplies" - provide enough detail so that the review committee knows you thought about what you really need to buy. You do not need to get quotes or bids at this stage, but they will be required as required by official purchasing guidelines if your proposal is funded. Again, no faculty salaries, buy-outs, or travel are allowed in projects.

Expenditure	Qty.	$/unit	Cost	Need/Justification
Employee salary [Ag Research Tech]	2080 hrs	$10/hr	$20,800	field technician
Employee benefits [Ag Research Tech]	1	7.65%	$1,592	field technician
sub-total			**$22,392**	
Student salary [Research Assistant I]	1000 hrs	$11.88/hr	$11,880	lab technician
Student benefits [Research Assistant I]	1	7.65%	$909	lab technician
sub-total			**$12,789**	
Materials & Supplies [CTiVd test kits]	1 kits	$800/kit	$800	viroid detection
Materials & Supplies [RNA isolation kits]	2 kits	$1,215/kit	$2,430	viroid detection
Materials & Supplies [RT-PCR beads]	2@100rxn	$550	$1,100	viroid detection
Materials & Supplies [disposable plastics]			$300	pipet tips, tubes, bags
Materials & Supplies [insect rearing boxes]			$100	boxes & growth media
sub-total			**$4,730**	
Other direct costs [________________]				
sub-total				
Total requested			**$39,911**	

Literature Cited (1 page)

Boccardo G, Beaver RG, Randles JW and Imperial JS. 1981. Tinangaja and Bristle Top, coconut diseases of uncertain etiology in Guam, and their relationship to Cadang-cadang disease of coconut in the Philippines. *Phytopathology* 71 (10): 1104-1107.

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Keese P, Osorio-Keese ME and Symons RH. 1987. Coconut tinangaja viroid: sequence homology with Coconut cadang-cadang viroid and other potato spindle tuber viroid related RNAs. *Virology* 162: 508-510.

Moore A, Jackson T, Quitugua R, Bassler P and Campbell R. 2015. Coconut rhinoceros beetles (Coleoptera: Scarabaeidae) develop in arboreal breeding sites in Guam. *Florida Entomologist* 98(3): 1012-1014.

Sullivan M, Daniells E and Robinson A. 2012. CPHST Pest Datasheet for *Coconut cadang-cadang viroid*. USDA-APHIS-PPQ-CPHST.

Wall GC and Wiecko AT. 1997. Detection of CTiVd and survey of Tinangaja on Guam. *Presented at the International Meeting on Detection and Management of Tinangaja and Other Coconut Pests*, Mangilao, GU, 21-23 Jan 1997.