Effects of Ultraviolet Light and Pheromone Release Rate in Trapping Coconut Rhinoceros Beetles, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), on Guam

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Abstract. Coconut rhinoceros beetle (CRB), *Oryctes rhinoceros* L., is a serious pest of coconut and oil palms throughout Southeast Asia and the Pacific. CRB was found on Guam in 2007 and, despite suppression efforts, has subsequently spread across the island. The CRB population on Guam is genetically different from other populations in Asia and the Pacific, and is considered a new invasive biotype (termed CRB-G). CRB-G is apparently resistant to *Oryctes rhinoceros* nudivirus, the preferred biocontrol agent for this pest. CRB populations are typically controlled with a combination of biocontrol, pheromone traps, and breeding site removal. A field trial was performed at six locations on Guam to test potential improvements to standard CRB pheromone trapping with oryctalure (ethyl 4-methyloctanoate). Two modifications were tested, 1) addition of ultraviolet light emitting diodes (UV LEDs), and 2) reduction of pheromone release rate. Addition of UV LED light sources to pheromone traps significantly increased trap catch by 2.85 times. Reduction in oryctalure release rate by up to an order of magnitude did not significantly change CRB capture rate. Further, when linear regression analyses of CRB trap capture rate as a function of pheromone release rate were conducted for traps with and without UV LEDs separately, only a very weak relationship between trap capture and oryctalure release rate was observed and only when a UV LED was present. Results suggest that addition of UV LED light sources to pheromone traps could improve detection trapping of CRB and that reduction of pheromone release rate could extend service life of lures without changing capture rate.

Key words: Oryctalure, UV-light, LEDs, CRB, population monitoring

Coconut rhinoceros beetle biology. CRB, *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae, Dynastinae), is a serious pest of coconut palm, *Cocos nucifera* L., and oil palm throughout Southeast Asia and on many Pacific islands. CRB damage to coconut palms is caused only by adults boring into crowns to feed on sap. Trees may be killed if this feeding activity damages the meristem. Although CRB damage does not always result in coconut tree mortality, the characteristic V-cut damage to palm fronds can adversely affect nut production and aesthetic value of

ornamental trees (Hinckley 1973, Zelazny 1979, Bedford 2013).

CRB larvae do not cause economic damage as they feed only on decaying vegetation. CRB breeding sites include dead standing coconut palms, fallen coconut logs, rotting coconut stumps, and decaying wood of many tree species (Bedford 1976, 2013), piles of compost, sawdust, manure piles and even in commercially packaged soil products (Moore et al. 2016). After feeding for a few days in the palm crowns, adults of both sexes aggregate at breeding sites where they mate, and females oviposit (Bedford 1980).

CRB aggregation at breeding sites is facilitated by the aggregation pheromone oryctalure, ethyl 4-methyloctanoate, which is produced by adult males (Hallett et al. 1995). Oryctalure is now the most commonly used chemical attractant in CRB pheromone traps replacing a previously used attractant, ethyl chrysanthemate, which was used in CRB eradication programs during the 1970s in Fiji (Bedford 1980) and Western Samoa (Maddison et al. 1973). Trapping with oryctalure is widely used for both management and ecological studies of CRB (Bedford 2013, Bessou et al. 2017).

CRB invasion history. CRB is native to Southeast Asia and the Philippines. CRB invaded islands in the Pacific and Indian oceans in two waves of movement. The first wave started in 1909 when CRB was accidentally transported from Sri Lanka to Samoa with shipment of rubber tree seedlings (Catley 1969) and it ended during the 1970s. All of the CRB range expansion during this period was south of the equator except for the invasion of the Ryuku Islands (Japan) starting in 1921 (Oshiro 1980) and invasion of the Palau Islands in about 1942 (Catley 1969). In Palau, there was a population explosion of CRB because WWII activities created abundant breeding sites. This resulted in about 50% coconut palm mortality overall, and total loss of coconut palms on some of the smaller islands (Gressitt 1953). The discovery and subsequent use of *Oryctes rhinoceros* nudivirus (OrNV) as a biocontrol agent on numerous Pacific islands led to major declines in pest beetle populations (Jackson 2009).

The second wave of CRB invasions started in 2007 with the discovery of CRB on Guam, followed by the invasion of Oahu (Hawaii), Port Morseby (Papua New Guinea), Guadalcanal, Savo and Malaita (Solomon Islands), and Rota (Commonwealth of the Northern Mariana Islands). Beetles in the second wave of invasions, termed ``Guam biotype'' or CRB-G, are genetically different from those in the first wave and are apparently resistant to biocontrol by OrNV (Marshall et al. 2017).

In theory, a CRB population can be eradicated from a newly invaded island by locating and destroying all active breeding sites and ensuring that the arrival pathway is blocked to prevent re-infestation. In practice, CRB eradication is difficult. There have been several CRB eradication attempts, but only one of these was successful. CRB was eradicated from Niuatoputapu Island, also known as Keppel Island, a tiny outer island of Tonga, only 16 square kilometers in area. Eradication was accomplished by a sanitation program which lasted 9 years following first detection in 1921 (Catley 1969).

CRB invasion of Guam. CRB was first detected in Guam in the Tumon Bay tourist hotel area in September 2007 (Moore 2018). A delimiting survey indicated that the infestation was restricted to only a small region of the island (<500 ha) (Smith and Moore 2008). Based on this information, an eradication attempt was launched. This project initially employed two tactics, 1) sanitation of active and potential breeding sites, and 2) mass trapping of adults. A third tactic, biological control, was

added in September 2009 after sanitation and mass trapping failed to suppress the increase and spread of the CRB population (Jackson 2010).

Sanitation. Sanitation is the most important tactic in any CRB eradication or control project. The objective is to find and destroy all breeding sites before adults are generated, thus halting reproduction and preventing all damage. The Guam eradication program employed four detector dogs trained to sniff out CRB larvae (phys.org 2009) in addition to visual inspections by project staff. Sanitation on Guam is more difficult than elsewhere because a significant proportion of the CRB population develops in detritus caught within the crowns of live coconut palms (Jackson 2010). Moore et al. (2015) found 446 immature CRB life stages (eggs, larvae or pupae) developing in the crowns of 121 live coconut palms. This habitat extension may be due to almost total absence of insectivorous birds and mammals as a result of heavy predation by the brown tree snake, *Boiga irregularis*. Despite sanitation efforts, CRB damage in central Tumon Bay remained high and the infestation spread to all parts of Guam by 2010, making eradication impractical.

Biocontrol agents. To date, attempts at population suppression using OrNV, the preferred biocontrol agent for CRB (Bedford 1986) have failed. OrNV was released on Guam in 2009, but produced no measurable results. Laboratory bioassays performed to investigate this failure indicated that the CRB population on Guam was tolerant to the isolate which was released and several other isolates available in cell culture. In addition, the CO1 DNA barcode for the Guam beetles was significantly different from that of other CRB populations in the Pacific (Marshall et al. 2017).

A second biocontrol agent, an entomopathogenic fungus, *Metarhizium majus*, was imported from the Philippines and field releases began in September 2011. Fungal spores were incorporated into CRB breeding sites where it established readily. An extensive island-wide postrelease survey conducted between October 30, 2014 and May 26, 2015 showed that between 10% and 38% of field collected CRB died from *M. majus* infection within 21 days after collection (Moore and Marshall 2015). This level of suppression was not sufficient to prevent CRB from killing many mature coconut palms following a population explosion triggered by abundant breeding material in the form of decaying vegetation left in the wake of Typhoon Dolphin which impacted Guam in 2015.

Pheromone traps. At the beginning of the project, panel traps baited with oryctalure were deployed in the Tumon Bay hotel area with the intention to suppress or decrease the CRB population. Observations showed that this mass trapping did not protect palms from new CRB damage. However, the program continued to operate an island-wide trapping network of about 2,000 traps to monitor the spread and growth of the CRB population. Island-wide trapping was discontinued when Typhoon Dolphin destroyed most eradication program traps in May 2015.

Laboratory and field studies suggest that the CRB-G population may not be highly attracted to oryctalure. In y-tube olfactometer trials, adult CRB were inconsistent in being attracted to oryctalure (Moore and Siderhurst, unpublished). In a study where 33 radio tagged CRB adults were released in the vicinity of multiple pheromone traps, only one beetle (3%) was caught in a trap containing oryctalure (Moore et al. 2017). In additional mark-release-recapture field trials where 567 CRB adults were released near the center of a pheromone trap grid (31 traps; 100 m spacing), only 64 (11%) were recovered

from the traps (Moore, unpublished).

However, contradictory data from Palau, indicates that CRB-G and a second haplotype, CRB-S (OrNV susceptible, CRB from the first wave of invasions), are equally attracted to pheromone traps baited with oryctalure (Adams 2019).

Records from island-wide detection trapping on Guam suggest that bucket traps containing oryctalure capture more CRB in the trap service period just before traps are refreshed with a new oryctalure release device. Presumably, the replaced release devices (depleted lures) emit less oryctalure than the new devices. Analysis of nearly 2,500 trap visit records shows that traps with depleted lures caught significantly more beetles. The capture rate for depleted lures was 0.220 beetles per trap-day in comparison to 0.092 beetles per trap-day for undepleted lures ($P < 0.001$, Welch two-sample t-test)(Moore 2012b). This observation led to the hypothesis that pheromone release rates from traps may be too high for optimal capture of CRB-G. Similar observations have been recorded for bark beetles, where pheromones are attractive at low release rates but become less attractive or repellent at high release rates (Borden 1996, Miller et al. 2005).

While conducting an y-tube olfactometer bioassays, we inadvertently observed that CRB appear to be attracted to light sources. This phenomenon has been previously investigated by Manjeri et al. (2011) who trapped CRB with both pheromone (oryctalure) traps and light traps. Unfortunately, Manjeri et al. (2011) do not provide details about what type of light traps were used or what wavelengths of light were emitted to attract CRB. Light trapping of CRB has also been used in Yemen where trapping was conducted throughout the year (Al-Habshi et al. 2006). However, in the South Pacific, CRB appears to be only occasionally attracted by light (Gressitt 1953, Luhukay et al. 2017). More broadly, light traps are known to attract a number of *Oryctes* beetles throughout the Middle East (Bedford et al. 2015). Moonlight has also been reported to affect the light trap catches of *Oryctes* beetles (Khalaf et al. 2011) and may also decrease the captures of non-light traps (Bedford 1975). Attraction of *Oryctes* beetles to light traps appears to vary based on the wavelength of light. Six light colors and two lamp types have been investigated with *O. agamemnon arabicus* with white light emitted from mercury lamps attracting more beetles than other light treatments (Al-Deeb et al. 2012).

Light traps that emit relatively large amounts of UV radiation are in general more attractive to nocturnal insects than those that emit other wavelengths (Matsumoto 1998, Shimoda and Honda 2013). Light-emitting diodes (LEDs) are now a low-cost and energy-efficient source of light for insect traps (Cohnstaedt et al. 2008, Shimoda and Honda 2013). LEDs typically produce light in a narrow 5-nm bandwidth ranging from 350-700 nm and have a cone of illumination that is dependent on the bulb shape. LEDs are particularly well-suited to field conditions as they are durable and function for up to several thousand hours.

Here we report on a field trial to determine if addition of UV LEDs and reduction of oryctalure release rate improved CRB trap captures at six locations on the island of Guam.

Materials and Methods

LED cage preference test. Effects of LED emission wavelength on the number of CRB captured were measured in a large outdoor cage (6 m x 6 m x 3 m) at the University of Guam Agricultural Experiment Station at Yigo. The two barrel traps (Iriarte et al. 2015) were placed adjacent to each other against one wall of

the screen cage with an oryctalure release device hung 10 cm above the point where the traps abutted. One trap was fitted with a white LED and the other was fitted with a UV LED. Approximately 20 adult CRB, collected from surveillance traps and held in peat moss, were released into the cage opposite the barrel traps on each evening between 2 January 2014 and 7 January 2014. CRB could access the traps either by walking or flying. Beetles were counted and removed from traps on the day following release. Further experimental details can be found in Moore (2014b).

Trapping sites and experimental conditions. CRB were captured in six trap lines on Guam located at the University of Guam Agricultural Research Station in Yigo (13.532° N, 144.873° E), the GICC Golf Course in Dededo, (13.519° N, 144.848° E), the Temple Baptist Church in Chalan Pago, (13.449° N, 144.777° E), the Leo Palace Golf Course in Yona, (13.416° N, 144.741° E), the Windward Hills Golf Course in Yona (13.381° N, 144.742° E), and the Chargalauf Farm in Inarajan, (13.250° N, 144.726° E) (Fig. 1). Trap lines were set perpendicular to prevailing winds at each location and the distance between adjacent traps was 20 to 50m. Traps were suspended at 3m above the ground from forked sticks.

Weather conditions during the experiment were mainly clear with occasional periods of rain and overcast skies. Over the trapping period, 19 April 2013-19 August 2013, the average temperature ranged from 26.6 to 30.4 °C with a mean of 29.2 °C (NOAA, Guam International Airport).

Traps. Standard double-vaned bucket traps (Hallett et al. 1995) were used to capture CRB in the field. Briefly, five-gallon plastic buckets were fitted with two corrugated plastic vanes or baffles set at 90° angles to each other. Holes were drilled in the bucket bottoms to release rainwater. Holes were also cut from the middle of each piece of corrugated plastic so that a pheromone lure could be hung between the baffles.

Figure 1. Trap line locations, from north to south, were located at the University of Guam Agricultural Experiment Station in Yigo, the GICC Golf Course in Dededo, the Temple Baptist Church in Chalan Pago, the Leo Palace Golf Course in Yona, the Windward Hills Golf Course in Yona, and the Chargalauf Farm in Inarajan. An on-line interactive version of this map is available at https://github.com/aubreymoore/CRB- trapimprovement/blob/master/map.geojson.

Pheromone lures. Oryctalure was obtained from ChemTica Internacional (Heredia, Costa Rica). The standard release rate lure consisted of oryctalure (~500 mg) sealed in a bubble pack with a plastic membrane to regulate the release rate. Preliminary work showed that rainwater entered the lure package making it difficult to accurately measure release rates. To solve this problem, each bubble pack was heat-sealed into a thin polyethylene bag, reducing the release rate by about 10%.

Reduced release rate lures were made by placing 200 µl of oryctalure into a 2 ml Eppendorf centrifuge tube with a 2 mm (5/64 inch) hole drilled in its top. The centrifuge tube was then placed in a bottle which acted as a rain and wind shield (Fig. 2). All lures were weighed before deployment and at the end of each trapping period to determine release rates.

Ultraviolet light sources. Two types of ultraviolet light emitting diode (UV LED) devices were used during the course of this study. The initial design used a battery pack of eight AA batteries to power four 10 mm UV LEDs (400-405 nm, purchased from www.suntekstore.com) with 1 k ohm resistor to reduce the current from 5.8 to 1.0 mA. The second design used a converted solar-powered lawn path light (various models) by replacing the standard white LED with a single UV LED which had been sanded to make it diffuse and omnidirectional. A photoelectric sensor in the second design turned on the UV LED between sunset to sunrise and turned it off during the day to conserve power. For construction details see Moore (2014a). Both devices were used in the course of the field study.

Field testing protocol. Effects of pheromone release rate and presence or absence of a UV light source on the number of CRB captured in double-vaned bucket traps was tested using a multi-factor balanced design. Six treatments were tested in the field trial: SL: standard oryctalure, RL: reduced release rate oryctalure, UV: UV LED light source, UV-SL: standard oryctalure plus UV LED light source, UV-RL: reduced release rate oryctalure plus UV LED light source, and Trap alone: trap without lure or light source (negative control). Six traplines were used (see site details above) with one trap from each treatment for a total of 36 traps .

Traps were serviced biweekly over a period of twelve weeks (19 April 2013-19 August 2013). During each trap service, pheromone lures were replaced and trapped CRB were counted and sexed. Treatments were assigned to traps using a randomization scheme which placed all treatments once at each trap site during the experiment.

Figure 2. Reduced release rate pheromone dispenser. A 2 mm hole in the tops of the Eppendorf centrifuge tube allows a slow release of the attractant oryctalure. The bottle shown acts as a rain and wind shield. This entire release device is placed within a bucket trap for field deployment.

Analysis. Total CRB trap captures were analyzed using the Fit Model platform of JMP Statistical Discovery Software, version 10.0.0 (SAS Institute 2012), with Lure (oryctalure release rate), Light (UV LED), and the interaction of Lure*Light

as model effects. The mean numbers of male and female CRB captured in traps were not significantly different by t-test so total CRB captured was used as the single response (dependent) variable. The factor (independent) variables were Lure (three levels: standard lure "SL", reduced lure "RL", and no lure) and Light (two levels: UV light "UV" and no light). Means comparisons were subsequently performed using either Tukey's HSD test (for Lure) or t-test (Light). Experimental difficulties prevented the collection of usable release rate data for the first four weeks of field trapping. Therefore, analysis of release rates was only conducted on data from weeks 5 through 12. Means comparisons between the release rates of the standard release rate lures and reduced release rate lures were performed using a t-test. Ordinary Least Squares regression was used to model CRB trap capture rates vs. pheromone release rates. The numbers of CRB captured with different LED lights in a large field cage were compared using an exact binomial test. All analyses of significance were made at the $P < 0.05$ level.

Results

LED cage preference test. Traps with white LEDs caught 15 beetles over the course of the experiment while traps with UV LEDs caught 100 beetles. This difference in trap capture was highly significant (binomial test; $P < 0.001$).

Field trial. Numbers of beetles captured by each trap type are summarized in Table 1. Overall, the number of CRB captured in traps was quite low. Most traps, regardless of type, were found empty at the end of each two-week trapping period. Both presence or absence of a UV light source and oryctalure release rates were found to have significant impacts on the number of adult *O. rhinoceros* trapped. While trap captures were dependent upon UV light as well as the release rate, release rate showed the more significant

Table 1. Beetle captures by trap type.

Trap type	Description (Trap with…)	Beetles trapped			Proportion of traps that caught beetles	Beetles/trap-day (mean ± SE)
		Males	Females	Total		
Trap alone	No lure or UV LED	0	0	0	0/36	0.0 ± 0.0
UV	UV LED but no lure	0	2	2	2/36	0.003 ± 0.002
RL	Reduced release rate lure	9	4	13	7/36	0.03 ± 0.01
SL	Standard release rate lure	11	9	20	10/36	0.04 ± 0.01
UV + RL	Reduced release rate lure and UV LED	18	20	38	12/36	0.07 ± 0.03
UV + SL	Standard release rate lure and UV LED	30	24	54	15/36	0.11 ± 0.03

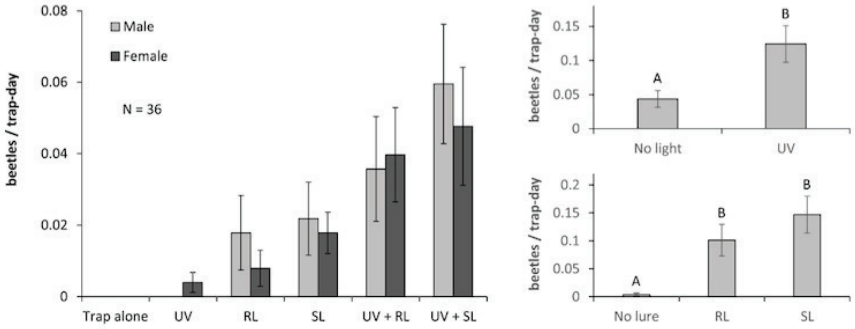

Figure 3. Capture rates (mean ± SE) of beetle caught in double-vaned bucket. UV = trap equipped with UV LED diodes, RL = trap with reduced release rate of oryctalure, SL = trap with standard release rate of oryctalure. Comparisons of mean trap capture between traps with and without UV light and between traps with different oryctalure release rates are shown at right. Bars with different letters indicate significantly different means (UV light: t-test, Lure: ANOVA, Tukey's HSD).

P value, with the interaction of light and release rate not being significant: Lure F = 8.77, *P* = 0.0002; Light F = 8.04, *P* = 0.0050; Lure*Light F = 1.77, *P* = 0.1737. With no multiplicative effect, trap capture appears to increase independently with the presence of oryctalure and UV light (Fig. 3): Tukey's HSD (letter denote significant differences in Lure means at *P* < 0.05): standard lure "SL" = A, reduced lure "RL" = A, and no lure = B, and t-test (letter denote significant differences in Light means at *P* < 0.05): UV light "UV" = A and no light = B. Unsurprisingly, traps without UV lights or oryctalure ("Trap alone") did not capture any CRB while traps equipped only with UV light caught only two beetles (Fig. 3). Overall, the addition of UV lights increased trap captures of CRB by 2.85 fold. Because only two beetles were trapped without lure, most of the increased trap captures are seen between traps with lights and oryctalure and those with only oryctalure. Despite the fact that the interaction of UV light and lure release rate was not significant, it seems likely that the lights and oryctalure increase trap captures synergistically. Interestingly, there was not a significant difference between the standard and reduced oryctalure treatments. There was no significant difference between the numbers of *O. rhinoceros* males and females trapped (t-test, *P* = 0.6211).

It should be noted that operational use of our UV LED devices following the experiment indicates that they are not reliable in the long term. Most failed within 3 months of initial use, requiring replacement.

Mean release rates for the standard and reduced release rate lures were 14.3 ± 0.4 mg/day and 1.41 ± 0.1 mg/day, respectively (*P*< 0.001), a roughly 10-fold difference in release rates. Linear regression of CRB capture rate as a function of pheromone release rate for traps with UV LEDs (Fig. 4) showed only a very weak relationship with a linear equation of y = 0.0182 + 0.0070x, an R2 of 0.111, but with the slope significantly different from zero (P = 0.005). Without UV LEDs, there was no relationship between trap capture and pheromone release rate (y = 0.0059 + 0.0015x, R2 = 0.036, slope not significantly different from zero, (P = 0.118)).

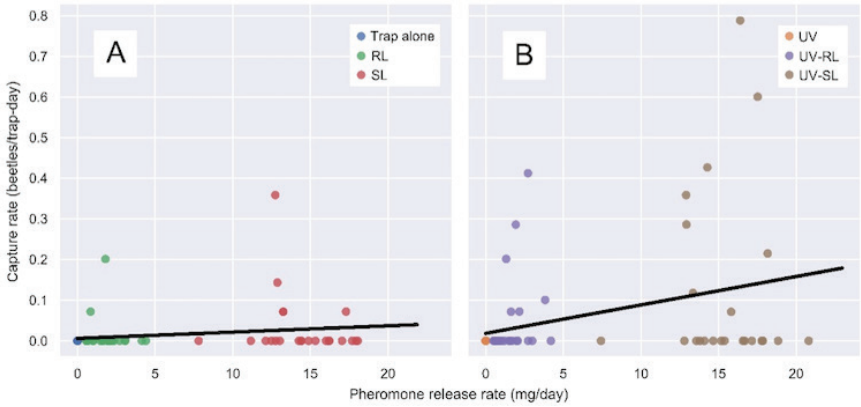

Figure 4. Capture rate as a function of oryctalure release rate for traps without (A) and with (B) ultraviolet light emitting diodes. UV = trap equipped with UV LED diodes, RL = trap with reduced release rate of oryctalure, SL = trap with standard release rate of oryctalure. Lines are ordinary least-squares fits. The equation for traps without UV LEDs is y = 0.0059 + 0.0015x; slope is not significantly different from zero (P = 0.118). The equation for traps with UV LEDs is y = 0.0182 + 0.0070x; slope is significantly different from zero (P = 0.005).

Discussion

Addition of UV LED light sources to pheromone traps baited with oryctalure significantly increased the capture of CRB. In our field experiment, traps equipped with UV LEDs trapped 2.85 as many beetles as those without LEDs. This strongly suggests that UV LED lights can be used to increase the effectiveness of trapping protocols using oryctalure as an attractant deployed as part of detection and population surveillance of CRB and may have potential uses in population suppression. As an example, UV LED lights have been incorporated into the standard oryctalure trap used in surveillance of the invasive CRB population on the island of Oahu, Hawaii (D. Oishi, pers. comm.).

UV light has been shown to attract a number of species of scarab beetles (García-López et al. 2011) and beetles more generally (Kato et al. 2000). Our cage experiment results show that traps with UV LEDs capture more beetles than traps with white LEDs. Phosphor-based white LEDs (like those used in this study) emit wavelengths across the visible spectrum, ranging from 400750 nm with peaks at roughly 450 nm and 575 nm. Therefore, we cannot say that CRB are preferentially attracted to UV light over other wavelengths but instead that UV LEDs are more attractive than broad-spectrum visible light. Previous CRB trapping using oryctalure and incandescent lights suggest that broad-spectrum light may be somewhat repellent as traps with incandescent lights and oryctalure captured fewer CRB than traps with oryctalure alone (Luhukay et al. 2017).

While trap captures with the standard lure were numerically higher than those with the reduced lure, the difference was not significant (Fig. 3). Likewise, traps without UV LEDs showed no dose-dependent relationship between oryctalure release rate and trap capture (Fig. 4A). This stands in contrast to the findings of Hallett et al. (1995) who found increasing trap captures with increased oryctalure

release rate up to 30 mg/day when trapping in in North Sumatra, Indonesia.

It is possible that this difference in observed pheromone response may be due to behavioral variations between the CRB populations. CRB found on Guam are considered to be a new invasive biotype, termed CRB-G. On Guam, CRB-G appear to be less attracted to oryctalure than has been reported elsewhere. This hypothesis comes in part from the observation that apparent CRB-G damage is much higher than beetle trapping rates would suggest, as reported by Jackson (2010).

In contrast to the data discussed above, which showed no dose-dependent CRB responses, traps with UV LEDs showed a very weak positive relationship between increasing trap captures and increased oryctalure release rate (Fig. 4B). Only two CRB were caught in traps with UV LEDs but without oryctalure demonstrating that the beetles are not strongly attracted to UV light alone. Field observations (A. Moore, pers. obs.) suggest that CRB are attracted to the general area of a trap by oryctalure with UV LED lights providing a short-range cue that increases trap captures. Increased trap captures resulting from the synergistic combination of pheromone attractants and light has been previously found in a number of beetles (Duehl et al. 2011, McQuate 2014). Interestingly, McQuate (2014) suggests that light is the long-range attractant while the pheromone functions as a short-range cue. Further study is needed to elucidate the behavioral mechanism underlying CRB attraction to traps containing both oryctalure and UV LEDs.

Results of this study suggest that the addition of a UV-LED light source to pheromone traps can improve the detection trapping of CRB. Additionally, reduction of oryctalure release rate can extend the field service life of lures.

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