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

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**Abstract**

The coconut rhinoceros beetle (CRB), *Oryctes rhinoceros* L., is a serious pest of coconut and other palms throughout Southeast Asia and on several Pacific Islands. Typically, CRB populations are 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 bucket trapping with oryctalure (ethyl 4-methyloctanoate). Two modifications to the standard trapping protocol were tested, 1) addition of ultraviolet light emitting diodes (UV LEDs), and 2) reduction of pheromone release rate. Addition of a UV-LED light source to pheromone traps significantly increased trap catch 2.85 fold over traps without a UV-LED. No difference was found between numbers of CRB caught with either of the oryctalure loading rates. There was no significant reduction in trap catch when the pheromone release rate was reduced by XX%. Results suggest that addition of a UV-LED light source to pheromone traps could improve detection trapping of CRB and reduction of pheromone release rate could extend the service life of lures.

**Key words:** Trapping, Pheromone, UV-light, *Oryctes rhinoceros*

**Introduction**

The coconut rhinoceros beetle (CRB), *Oryctes rhinoceros* L.(Coleoptera: Scarabaeidae, Dynastinae), is a serious pest of coconut trees, *Cocos nucifera* L*.*, and other palms throughout the Pacific and Southeast Asia. Adult beetles damage and sometimes kill palms when they bore into crowns of palms to feed on sap. Palms die when boring and feeding activities kill the apical meristem. Although CRB damage does not always result in coconut tree mortality, the characteristic V-cut damage to palm fronds can adversely affect the nut production and the aesthetic value of ornamental trees ([Hinckley 1973](#_2jxsxqh), [Bedford 2013](#_4d34og8), Zelazny 1979).

CRB damage to coconut palms is caused only by adult CRB feeding in crowns. In contrast, larvae cause no economic damage as they feed on decaying vegetation at breeding sites, which include dead standing coconut palms, fallen coconut logs, rotting coconut stumps, and decaying wood of many tree species ([Bedford 1976](#_tyjcwt), [2013](#_4d34og8)). Breeding sites are also found in piles of compost, sawdust, and manure where these materials are available. After feeding in the crowns of palms, adults of both sexes return to breeding sites where they aggregate and 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](#_35nkun2)).

Oryctalure is the primary attractant used for trapping detection of CRB. Oryctalure replaced a previously used attractant ethyl chrysanthemate, which was used in the failed CRB eradicate attempted on two islands in Fiji from 1971 through 1974 ([Bedford 1980](#_3dy6vkm)). Trapping with oryctalure is now widely used for both management and ecological studies of CRB (Bedford 2013, Bessou et al. 2017). Oryctalure has been shown to increase trap captures in a dose dependent fashion up to release rates of 30 mg/day ([Hallett et al. 1995](#_35nkun2)). Empty fruit bunches (host plant material) appear to synergize oryctalure as traps containing both increase CRB captures 2 to 4 times larger than with the pheromone alone ([Hallett et al. 1995](#_35nkun2)).

CRB was first detected in Guam in the Tumon Bay tourist hotel area in September, 2007. A delimiting survey indicated that the infestation was restricted to only a small region of the island (<500 ha) and an eradication project was launched ([Smith and Moore 2008](#_1pxezwc)). The project relied on mass trapping using pheromone traps to capture adults and sanitation to remove rotting vegetation used as breeding sites.

Despite these efforts, CRB damage in central Tumon Bay remained high and the infestation spread to all parts of Guam by 2010, making eradication impractical at that time. Attempts at population suppression using *Oryctes nudivirus* (OrNV), the preferred biocontrol agent for CRB ([Bedford 1986](#_1t3h5sf)), also failed. It has recently been determined that the Guam CRB population is genetically different from other populations in Asia and the Pacific, and it is considered to be a new invasive biotype of CRB, named CRB-G, that is apparently resistant to biocontrol by OrNV ([Marshall et al. 2015](#_4i7ojhp)). In addition to being resistant to all currently available isolates of OrNV, it appears that the CRB-Guam biotype behaves differently. CRB breeding sites are commonly found in coconut palm crowns on Guam ([Moore et al. 2015](#_1ci93xb)), but arboreal breeding sites are found only occasionally in other areas where the beetle occurs.

Records from widespread 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 devise. This observation led to the hypothesis that loading rates, and therefore release rates, from traps may be too high for optimal capture of CRB. This hypothesis is supported by research on bark beetles, in which pheromones may be attractive at low release rates but become less attractive or repellent at high release rates (Borden 1996, Miller et al. 2005).

While conducting an attraction bioassay with CRB, we inadvertently observed that CRB appear to be attracted to different light sources. This phenomena 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 wavelength of light. Six light colors and two lamp types has been investigated with *O. agamemnon arabicus* with white light emitted from mercury lamps attracted 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.

Herein, we report on a field trial to determine if UV LEDs and oryctalure loading rates improved CRB trap captures at six locations on the island of Guam.

**Materials and Methods**

**Trapping sites and experimental conditions**

CRB were trapped at six locations on Guam: the University of Guam Agricultural Research Station in Yigo (13.532444**°** N, 144.873333**°** E), the GICC Golf Course in Dededo, (13.5198**°** N, 144.84877**°** E), the Temple Baptist Church in Chalan Pago, (13.449677**°** N, 144.777665**°** E), the Leo Palace Golf Course in Yona, (13.41695**°** N, 144.7413**°** E), the Windward Hills Golf Course in Yona (13.38139**°** N, 144.74202**°** E), and the Chargalauf Farm in Inarajan, (13.250861**°** N, 144.726708**°** E) (Figure 1). Trap lines were set perpendicular to prevailing winds at each location and the distance between adjacent traps was 20 to 50 m. Traps were suspended at 3 m 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, average temperature ranged from 26.6-30.4 ℃ with a mean of 29.2 ℃ (NOAA, Guam International Airport).

**Traps**

Standard double-vaned bucket traps ([Hallett et al. 1995](#_35nkun2)) were used throughout this study. Briefly, five gallon plastic buckets were fitted with two corrugated plastic vanes or baffles set at 90o angles to each other. Holes were drilled in the bucket bottoms to release rain water. Holes were also cut from the middle of each piece of corrugated plastic so that a pheromone lure could be hung between the baffles.

**Pheromone lures**

Oryctalure was obtained from Chemtica (Heredia, Costa Rica). The standard loading lure consisted of oryctalure sealed in a bubble pack with a plastic membrane to regulate the release rate. Preliminary work showed that rain water entered the bubble packs making it difficult to accurately measure the 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 loading 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. All lures were weighed before deployment and after pick up in order 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. Design and fabrication details are available online (Moore 2013).

**Field testing protocol**

The effects of both lure loading rate and UV light 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: 1) standard oryctalure loading (SL), 2) reduced oryctalure loading (RL), 3) UV LED light (UV), 4) standard oryctalure loading and UV LED (UV + SL), 5) reduced oryctalure loading and UV LED (UV + RL), and 6) vaned-baffle bucket traps with no attractant (negative control). 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.

**Analysis**

Means comparisons between the release rates of the standard loading lures and reduced loading lures were performed using a t-test. 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). All analyses of significance were made at the *P <* 0.05 level.

**Results**

Mean release rates for the standard and reduced loading lures were 14.32 mg/day and 1.41 mg/day, respectively (*P* < 0.001) (Figure 2). Both UV light and oryctalure release rate 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 *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 (Figure 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 (Figure 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 seem between traps with lights and oryctalure and those with only oryctalure. Dispite 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 in the number of male or female *O. rhinoceros* trapped (t-test, *P* = 0.6211).

**Discussion**

UV LED lights significantly increased the captures of CRB in our study. This strongly suggests that UV LED lights could 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).

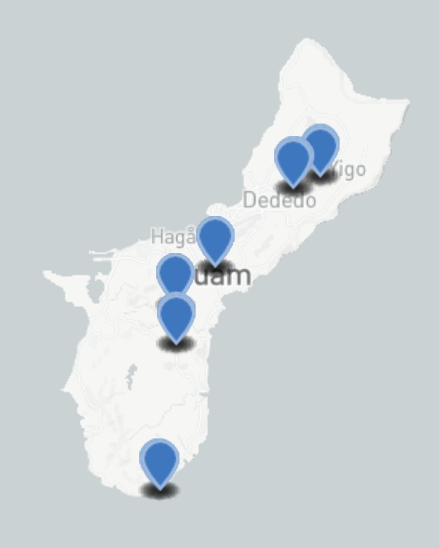
Traps without UV LEDs showed no dose dependent relationship between oryctalure release rate and trap capture (Fig. regression). This stands in contrast to the findings of Hallett et al. (1995) who found increasing trap captures with increased oryctalure release rate up to 30 mg/day when trapping in in North Sumatra, Indonesia. It is possible that is difference in observed pheromone response may be due to behavioral variations between the 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 from the observation that apparent CRB-G damage is much higher than beetle trapping rates would suggest (ref? help Aubrey, I think I remember you saying this).

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).

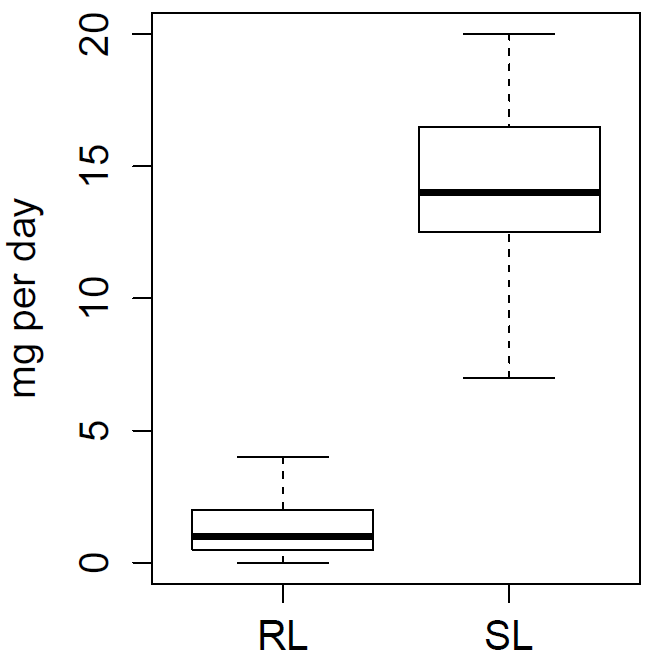
* How this compares to other beetles attracted to UV light
* How this compared to other release rate studies
* How this might help detection trapping in the future
* Synergistic?

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**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-trap-improvement/blob/master/map.geojson>.



**Figure 2.** Mean release rates for the standard and reduced loading lures. RL = reduced loading of oryctalure, SL = standard loading of oryctalure. Release rates were significantly different (t-test, *P* < 0.001).

C:\Users\ms826\AppData\Local\Microsoft\Windows\INetCache\Content.Word\Figure 1 CRB mark 2-2.tif

**Figure 2.** Capture rates (mean ± SE) of beetle caught in double-vaned bucket. UV = trap equipped with UV LED diodes, RL = reduced loading of oryctalure, SL = standard loading of oryctalure. Comparisons of mean trap capture between traps with and without UV light and between traps with different oryctalure loading are shown at right. Bars with different letters indicate significantly different means (UV light: t-test, Lure: ANOVA, Tukey’s HSD).

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