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**Judas Beetles: Discovering Cryptic Breeding Sites by Radio Tracking 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 trees and other palms throughout Southeast Asia and on several Pacific Islands. Adults damage and sometimes kill palms when they bore into the crown to feed. In contrast, larvae feed only on dead plant material at breeding sites. Typically CRB populations are controlled with a combination of biocontrol, pheromone traps, and breeding site removal. A field trial was performed at two locations on Guam to test the feasibility of using radio-tagged adults to discover cryptic breeding sites. Of 33 radio-tagged beetles that were released, 19 were successfully tracked to landing sites in five different microhabitats, 11 of which were considered to be active or potential breeding sites. The remaining 14 beetles were lost when they flew beyond the range of our receivers. None of the radio-tagged beetles were caught in the numerous pheromone traps present at the release sites. Percent emergence weight (%EW) varied significantly by the microhabitat to which CRB were tracked. When microhabitats were further grouped, the difference in mean %EW between the arboreal (74 ± 2%) and the soil-associated (82 ± 3%) groups were found to be highly significant. The %EW for CRB that were successfully located (78 ± 2%) and those that were lost (72 ± 2%) also differed significantly. Tracking CRB in this manner shows good promise as a method to identify cryptic breeding sites, which could then be treated, removed, or destroyed.

**Key words:** radio tracking, *Oryctes rhinoceros*, breeding sites,

**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 activity kills 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 aesthetic value of ornamental trees ([Bedford, 2013](#_ENREF_5" \o "Bedford, 2013 #12); [Hinckley, 1973](#_ENREF_9" \o "Hinckley, 1973 #21)).

CRB damage to palms is caused almost exclusively by adult CRB feeding in coconut tree crowns, while larvae causing little or no economic damage as they feed on decaying 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](#_ENREF_3" \o "Bedford, 1976 #22); [2013](#_ENREF_5" \o "Bedford, 2013 #12)). Breeding sites are also found in piles of compost, sawdust and manure where these materials are available. When CRB breeding sites are abundant following damage from typhoons, war, or large scale agricultural operations, a self-sustaining positive feedback loop may be initiated where large numbers of CRB adults kill large numbers of palms creating new breeding sites which generate even more CRB. This worst case scenario was observed in the Palau Islands when CRB arrived near the end of World War II. A CRB population outbreak was fueled by availability of abundant breeding sites in the form of trees killed by military activities. Fifty per cent of coconut palms were killed by CRB throughout the Parlay Islands and some of the smaller islands lost all of their coconut palms (Gressitt, 1953).

After feeding in the crowns of palms, adults of both sexes return to breeding sites where they mate and females oviposit. Location of breeding sites is facilitated by an aggregation pheromone produced by adult males (Hallett et al., 1995). The feeding-mating-oviposition cycle generally repeats multiple times throughout the lifetime of adult beetles (Gressitt, 1953). Vander Meer (1987) developed a body mass index, per cent emergent weight (%EW), which is strongly correlated with the physiological and behavioral status of CRB adults.

CRB first was 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 of the island (<500 ha) and an eradication project was launched (Smith et al., 2008). The project relied on mass trapping using pheromone traps to capture adults, and sanitation to remove rotting vegetation used as breeding sites. In addition, four detector dogs were trained to assist in finding breeding sites on the ground by sniffing out CRB grubs.

Despite these efforts, CRB damage in central Tumon Bay remained high and the infestation spread to all parts of Guam by 2010 making eradication unfeasible. Attempts at population suppression using *Oryctes nudivirus* (OrNV), the preferred biocontrol agent for CRB (Bedford, 1980; Bedford, 1986), 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 which has escaped from biocontrol by OrNV (Marshall et al., 2015). 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 but only occasionally elsewhere (Moore et al., 2015) and pheromone traps baited with oryctalure are not very attractive, catching only a small proportion of beetles in the vicinity of each trap (Moore, unpublished).

Eradication of CRB from an island is difficult once this pest has become established. Currently available pheromone traps, although useful for detection and surveillance, are not attractive enough to provide significant population suppression in mass trapping operations. Mass trapping coupled with sanitation during 1971 through 1974 failed to eradicate CRB on two islands in Fiji (ref needed). The only proven tactic for eradication is a vigorous sanitation program which discovers and destroys all active and potential breeding sites. The single successful CRB eradication to date occurred on the tiny (36 km²) Niuatoputapu Island (also known as Keppel Island), which lies between Samoa and Tonga, using sanitation alone (Gressitt, 1953; Catley, 1969).   Given the importance of finding and destroying breeding sites in CRB eradication, and the inherent difficulty of locating breeding sites, which are often cryptic and are found in a wide range of locations ([Bedford, 2013](#_ENREF_5" \o "Bedford, 2013 #12); [Hinckley, 1973](#_ENREF_9" \o "Hinckley, 1973 #21)), there is a pressing need to develop detection methods to reliably find these sites.

One such detection method in the use of detector dogs. As part of the CRB eradication program on Guam four detector dogs were trained and employed for the first time. The dogs were trained to sniff out CRB grubs and it was hoped that they would facilitate the discovery of cryptic breeding sites in the final stages of eradication. The detector dogs were a very expensive component of the Guam CRB eradication program and they were retired after two years of service when it became evident that eradication was no longer feasible.

Alternatively, predators/parasitoids or conspecifics of pest insects have evolved superior sensory systems to find either prey or mates in a complex natural environment and are the most adequate agents to detect a species. Following this idea, a novel way to detect pest insects in the wild has been recently discovered. Swink (*et al.*) described the use of the predatory wasp *Cerceris fumipennis*, a natural predator of different beetles in the Buprestidae family, to specifically monitor the emerald ash borer. Although this biological control agent succeeded in capturing a large number of beetles, *C. fumipennis* could not serve as a selective control agent as it collected samples of 52 different species in 11 different genera ([Swink et al., 2013](#_ENREF_13" \o "Swink, 2013 #35))). An obstacle to using conspecifics is the necessity to have the capability of following the marked individual. This problem is thoroughly addressed by using radio telemetry to investigate insect populations and behavior. Rink and Sinsch have utilized radio telemetry to study population migration and connectivity of the stag beetle *Lucanus Cervus* in order to define conservation efforts for the species ([Rink, 2007](#_ENREF_12" \o "Rink, 2007 #36))). Similarly, Beaudoin-Olliver (*et al.*) has implemented radio telemetry to successfully describe the flight behavior of the species *Scapanes australis* of the Dynastinae subfamily, to which CRB belongs ([Beaudoin-Ollivier et al., 2000](#_ENREF_2" \o "Beaudoin-Ollivier, 2000 #19)). In both of these cases, radio telemetry proved to be able to successfully track individual beetles, elucidating its potential use in the control of insect pests with conspecifics.

The semio-chemical communication adult CRB utilize to find mates in breeding sites provide a prime opportunity to locate cryptic breeding sites. This chemical communication signaling can be exploited by using radio telemetry to follow adult CRB that are seeking these cryptic breeding sites. This study seeks to develop a control mechanism that uses laboratory-reared CRBequipped with miniature radio-tracking devices to identify cryptic breeding sites, which could then be treated, removed or destroyed. In this article, we report results of a field trial intended to test the concept of following radio-tagged CRB to cryptic breeding sites as an alternative to using detector dogs.

**Materials and Methods**

**Release sites and experimental conditions**

Tagged CRB were radio tracked after release at two locations on Guam: the University of Guam Agricultural Research Station in Yigo (13°31'56.8"N 144°52'24.0"E) and the War in the Pacific National Historical Park in Asan (13°27'57.5"N 144°42'39.4"E). The Yigo site is an inland agricultural experiment station farm bordered by residential areas and uncultivated forest areas that include coconut palms along with other trees. At the time of the field trial, most coconut palms on the station were showing signs of CRB damage. The release site (144.872750º E, 13.531333º N) was in the middle of an uncultivated field. Beetles were released in the vicinity of three types of pheromone traps baited with oryctalure: standard vaned bucket traps (n=3 traps) (Hallett et al. 1995), barrel traps made from 45 gallon drums (n=31), and DeFence traps made from plastic netting that entangles CRB (n=4). Asan Beach National Park is roughly triangular with the ocean bordering one side, coastal wetlands on another, and forested hillside on the third. The park itself is a large, open, grassy field and includes with coconut palms on the edges, many of which displayed CRB damage at the time of the study. The release site (144.708537º E, 13.473904º N) was at the middle of a large grassy field. ASAN TRAP INFO NEEDED. Thus, both sites feature relatively accessible terrain that provides a variety of potential breeding sites as well as adult food sources.

Weather conditions during the experiment were mainly clear with occasional periods of rain and overcast skies. On release dates, August 8 to August 14, average temperature ranged from 27°C to 29°C while relative humidity was 80% to 88%. Beetles were generally tracked under clear skies with the exception of August 9 during which light showers occurred.

**Collection, selection and preparation of test insects**

CRB used for radio tracking were wild-caught in bucket traps containing oryctalure and collected within one week of capture. These beetles were placed in tubs containing moist peat moss, fed fresh banana slices and allowed to rest for at least three days.

Only CRB capable of flight were selected for radio tagging and release. After the rest period, captured beetles were flight tested at least one day prior to experimentation. The flight test chamber consisted of a large 121 L lidded garbage container. Within the chamber, about 30 beetles were placed in a smaller open metal bowl half filled with moist peat moss atop an upside down 19 L bucket. Beetles could only exit the smaller open container by flying out of it; therefore, any beetle found on the bottom of the flight chamber container the next morning was considered flight-capable. Flight capable CRB were transported and stored until release in lidded plastic bins approximately 45 cm by 30 cm by 18 cm containing 4 to 6 inches of damp peat moss.  Because not all beetles flew when first taken into the field, some beetles remained in storage for up to six days.

Flight-capable beetles were marked with a unique four-digit code engraved on one elytrum using a laser engraver (Fenix Flyer, Synrad Inc., Mukilteo, WA, United States). The sex, mass and elytral dimensions of each beetle were recorded.

A transmitter was glued to the pronotum of each beetle using a hot-melt glue gun (Fig. S1). Prior to transmitter attachment, the beetle pronotum was abraded with sandpaper to improve adhesion.

**Tracking equipment**

Beetles were tracked using a radio receiver (model R410) equipped with a three-element folding Yagi antenna (model 13863) (Fig. S2). Two receivers operated in the 148.641 to 148.992 MHz frequency band and two operated in the 164.032 to 164.409 MHz band.

Transmitters (A2414) had a maximum battery life of 45 days with a warranty guarantee of 22 days. Each transmitter had a mass of approximately 300 mg and was secured with approximately 250 mg of adhesive. Transmitter frequencies for individual CRB were recorded in conjunction with beetle identification numbers. All radio tracking equipment was purchased from Advanced Telemetry Systems, Isanti, Minnesota.

In addition to radio tracking equipment, handheld GPS units (Garmin Oregon 650, Salem, OR) were used to record locations where beetles were found or point of signal loss for each beetle.

**Beetle release and tracking procedure**

Beetles were transported to release sites in plastic storage bins. The lid of the bin was removed at dusk (roughly 19:30) and the container was closed at roughly 21:30. Once the containers were opened, CRB activity was carefully monitored using an infrared camera. Observation under the infrared camera revealed that beetles thermal profile would change just prior to flight, and thermally active beetles observed emerging from the peat moss were briefly viewed under red light to record the identification number and determine the frequency of the radio transmitter. Though nearly all beetles flew independently, several beetles that had not yet flown by the end of experimentation were encouraged to flight by removing them from the peat moss and throwing them into the air to facilitate takeoff.

CRB were pursued on foot following release and were tracked until a landing site was determined or until the transmitter signal was lost. In either case, a waypoint was recorded at the landing site or the last point of signal reception using a GPS unit.

Landing sites were visited on the following morning, and attempts were made to more precisely determine the location of each beetle. Beetle locations were monitored over several days, and beetles and or transmitters were recovered when possible at the end of the experiment. CRB and transmitters were successfully recovered by digging up beetles that buried into soil or compost; however the locations of CRBtracked to coconut crowns could not be as exactly determined due to the density of the frond foliage.

**Analysis**

In assessing the flight patterns of beetles for trends between sex and size, percent emergence weight (%EW) was calculated as an additional consideration. Percent emergence weight describes CRB mass at the time of measurement relative to its estimated mass upon emergence. This value can be estimated based upon a linear equation relating elytral measurements and emergence weight (Vander Meer and Mclean, 1975). This value is significant in data analysis because %EW reflects the present life stage of a beetle and how much stored energy it has available; CRB emerge at their heaviest weight and gradually lose weight over their lifespan.

The GPS coordinates of the release sites at both experimental locations and of each CRB landing site were displayed as X,Y data on ArcMap Version 10.0. The CRB flight distance was calculated by using the Point Distance tool on ArcMap Version 10.0.

-stats (Matt)

**Results**

The tagging and radio tracking of CRB in this study led to the successful location of multiple cryptic breeding sites at both experiment sites. CRB were most active from approximately 19:30 to 21:00, and flight activity did not appear to be heavily influenced by the prevailing weather conditions. Transmitters did not inhibit the flight mechanics of CRB to an observable degree. Over the course of experimentation, it was observed that beetles employed thermogenesis in flight muscles directly prior to flight, allowing a reliable prediction to be made as to which beetles were about to fly by detecting thermal radiation with an infrared camera.

A total of 33 out of 34 beetles tagged for release flew during the course of this study. Of the 33 beetles that flew, 19 were successfully tracked to landing sites (Figure 1). The % EW for CRB that were successfully located, 78 ± 2%, and for CRB that were lost, 72 ± 2%, differed significantly (t-test: *P* = 0.021). However, EW (*P* = 0.822) and weight (*P* = 0.510) did not differ between CRB that were successfully tracked or lost after release. Additionally, there were no differences in the numbers of male and female CRB that were successfully located or lost (Fisher’s: *P* = 1.000).

No relationship was found between the distance beetles moved from the release point and beetle EW (*R2* = 0.0686), %EW (*R2* = 0.0462), or weight (*R2* = 0.0465). There was no difference in the mean distance beetles moved at the two experimental sites, Yigo, 276 ± 42 m, and Asan, 215 ± 57 m (*P* = 0.408). Additionally, no differences were found between the mean distances male (254 ± 44 m) and female (233 ± 61 m) beetles moved (*P* = 0.778).

Landing locations of CRB were categorized by microhabitats described as other trees, coconut crown, traps, base of trees, or soil unassociated with trees or traps. Percent emergence weight varied significantly by the microhabitat to which CRB were tracked (Figure 2a., ANOVA: *F* = X.XXX, *P* = X.XXX). Microhabitats of CRB were further clustered as arboreal (> 1 m above ground) or terrestrial destinations (< 1 m above ground) (Figure 2b.). When microhabitats were grouped as either arboreal or soil-associated, the difference in mean %EW between the groups, arboreal, 74 ± 2%, soil-associated, 82 ± 3%, was found to be highly significant (t-test: *P* < 0.001). In addition, while emergence weight (EW) was significantly different between arboreal (6.5 ± 0.4 g) and soil-associated (4.9 ± 0.5 g) microhabitats (t-test: *P* = 0.020), there were no differences in weight (*P* = 0.160) or distance travelled (*P* = 0.908) between these microhabitat groupings. The numbers of male and female beetles did not vary between arboreal and soil-associated microhabitats (Fisher’s: *P* = 1.000).

Arboreal destinations were most commonly the crowns of coconut palms damaged by bore holes; however, beetles also landed in the branches of other species of trees. For example, an upper branch of a large breadfruit tree damaged in a recent typhoon was the final destination of one beetle. Upon investigation, several other beetles and grubs were found in the rotting limb along with the radio transmitter. In another instance, two beetles flew to the crown of the same highly damaged coconut tree independently of one another.

In soil associated landing site, CRB tended to bury into the soil upon landing at depths up to approximately 15 centimeters. Typically, these sites were at the base of a tree. Four out of five of these landing sites were at the base of coconut palms, though CRB also landed in less predictable locations. For example, one beetle landed beneath a trailer parked on a grassy lawn in a residential area surrounding Triton Farm. In two other examples of particular interest, one beetle was found beneath a CRB barrel trap baited with oryctalure at each experiment site. Other beetles and larvae were found also beneath one of these traps.

**Discussion**

This study was successful in tracking CRB to cryptic breeding sites at two locations on the island of Guam. The two areas where CRB were tracked differed both in topography and vegetation and the effective location of tagged beetles in these different environments shows promise for the applicability of this technique in the varied habitats were CRB infestations may occur. Out of 33 released CRB, a total of 19 were followed to final landing sites, resulting in a 58% tracking rate. This comparatively high retrieval rate required an input of approximately 30 minutes per CRB immediately after release and about the same amount of time on the following day. . The tracking of CRB to an approximate location during the night followed by a more precise pinpointing during the daytime proved to greatly facilitate the retrieval of released CRB.

**ANECTDOTAL LOCATIONS**

Although a majority of the released CRB were successfully tracked to discrete locations, 14 CRB were lost presumably due to out-of-range flights. Interestingly, those CRB that flew out of range had statistically significantly lower percent emergence weights than those that stayed within the detection range of the radio devices, suggesting that lighter CRB further from initial release site, presumably in search of food sources.. State average %EW. This observation raises the ability to minimize the loss of CRB while radio tracking. Prior to release, CRB must be fed to above 70 percent of their emergence weight to ensure that the individuals will remain within the detection radius. State average %EW. The distance that found CRB flew from release site had no statistically significant correlations with percent emergence weight. However, the distance flown for all 33 CRB that were released, both lost and found, could correlate with percent emergence weight if the distance of the lost CRB were determined.

Moreover, percent emergence weight of released CRB had a strong association with the microhabitats in which tagged CRB were found. Of the 19 retrieved CRB, 11 landed arboreal microhabitats whereas 8 landed in soil-associated microhabitats. The CRB that landed in the arboreal microhabitats had a statistically significantly lower percent emergence weight than those CRB that landed on soil-associated microhabitats, 74.43% compared to 82.73% respectively. It has been noted that adult CRB spend their time either feeding on the crown of palms or breeding in either soil or compost piles (Zelazny 1975)[[1]](#endnote-1). As CRB alternate between these microhabitats, individuals fluctuate in their percent emergence weight making it possible to determine the behavioral pattern that CRB will engage in by noting their percent emergence weight (Source). CRB at a higher percentage of their emergence weight will very likely refrain from further feeding and fly in search of breeding sites whereas CRB at a lower percentage of their emergence weight will likely forage in search for food. Therefore, it is not coincidental that the CRB that landed in ground microhabitats, associated with breeding, had statistically significantly higher average percentage emergence weights than those that landed in palm crowns, associated with feeding sites. This characteristic of the CRB life cycle makes this tracking method specific and controllable In order to increase the probability that CRB fly directly to breeding sites, the individuals must be fed to above 80 percent of their emergence weight. In doing so, monitoring and eradication teams can improve the likelihood that the released CRB will not lead them to feeding sites rather than breeding site, which will increase the effectiveness of CRB control methods. We speculate that heavier CRB landed on lower microhabitats due to the comparative inability to reach higher altitudes than the ones reached by lighter CRB. However, all of the released CRB flew several meters vertically into the air before displacing horizontally. If heavier CRB could not reach altitudes as high as lighter CRB, then takeoff would have been notably different between these two groups. Since hindered vertical displacement was not observed during CRB takeoff, it is reasonable to conclude that the difference in landing microhabitats was most likely due to the percent emergence weight of released CRB.

In assessing the final landing locations of beetles that were tracked successfully, it is important to note the varied and cryptic nature of these sites. For example, the breeding site found in the top of a breadfruit tree was well hidden within the branches and approximately twenty feet above the forest floor. This example demonstrates a site that would be nearly impossible to find with current methods. Breeding sites discovered in other unusual locations such as beneath barrel traps or buried in the soil also demonstrate how this tracking with conspecifics provides an advantage in such situations that cannot be rivaled by present tracking techniques.

METHODOLOGY

The use of radio telemetry to monitor flying species is generally constrained by the weight of radio transmitters (Robinson *et al.* 2009, Cochran *et al.* 2004, Thorup *et al.* 2007). This limitation is especially true when monitoring flying insects since a small increase in weight may severely hinder flight behavior (Source). In recent years, though, the gradual miniaturization of transmitters has circumvented this obstacle allowing for more precise monitoring of flying insects (Sato Maharbiz 2010). One of the factors determining the feasibility of this study was whether or not adult CRB could fly undisturbed with the attached radio transmitters. Adult CRB are excellent fliers and can exert force much larger than their body weight when fighting and boring, so it was reasonable to expect that the miniature radio transmitters would have little to no effect on CRB flight capability. As expected, the flight capability of CRB was seemingly unaffected by the extra weight of radio transmitters. Each radio transmitter amounted to between 5.04% and 9.72% of the CRB weight at the time of release, and there was no correlation between the increased percentage weight and the single flight distance of CRB indicating that CRB could fly carry the extra burden of the radio transmitters. Note about whether it went over 100% It is also important to note that the 14 CRB were lost did present not statistically significant differences in the radio transmitter percentage weights compared to the CRB that stayed in range. These observations are consistent with other studies monitoring members of the Scarabaeidae family which found that radio transmitters did not noticeably affect beetle flight capabilities (([Beaudoin-Ollivier et al., 2000](#_ENREF_2); [McCullough, 2013](#_ENREF_11)), McCollough 2006, (Rink, 2007[Rink, 2007](#_ENREF_12))). Also, the duration of commercially available radio transmitters (10-14 days) is appropriate for this type of CRB monitoring. However, the battery life of the transmitters must guide monitoring protocol timelines. CRB should be pinpointed to a final location within 2-3 days after initial release to prevent the loss of CRB due to battery drainage.

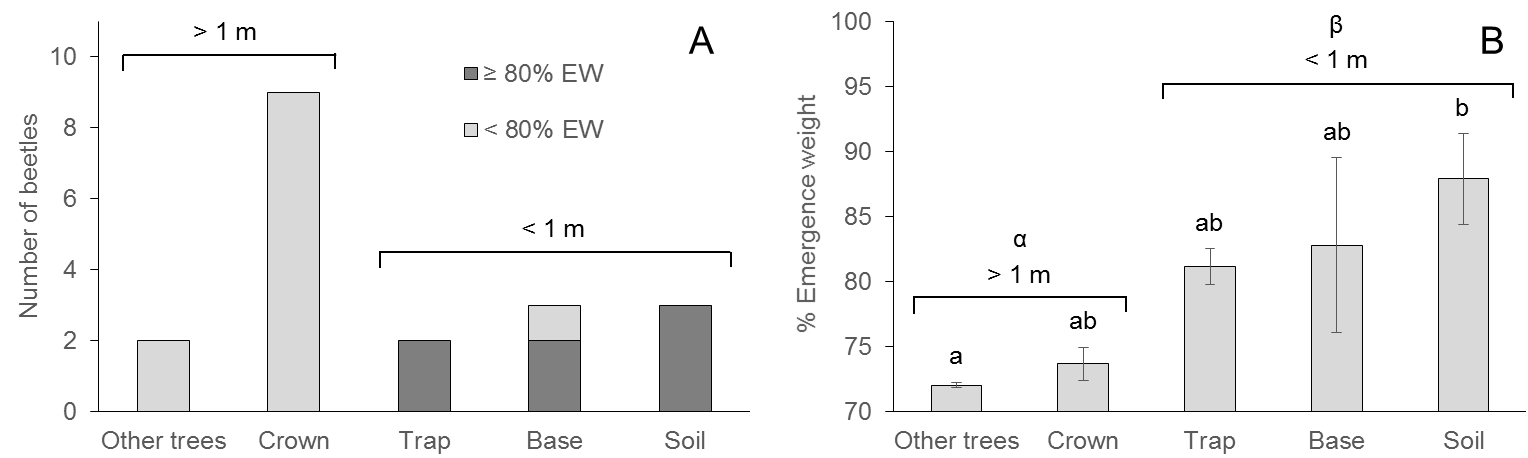
Another important factor to consider is the distance over which CRB can be monitored. Radio telemetry monitoring typically covers only short to medium displacement distances usually limiting the applications of the technology (Robinson *et al.*2009). The radio devices employed in this study had an effective range of localization that varied with topological conditions. In this study, the range of detection was appropriate for monitoring since the overall CRB flight distance from release sites to landing sites ranged from 52.8 meters to 564.6 meters. This range also roughly delineates a radius for breeding site discovery from released CRB; the detector CRB must be released no further than approximately 500 meters from breeding sites. This might present difficulties for eradication teams since the breeding sites in question occur in cryptic locations presumably unknown to those searching for them. The relatively short detection radius of the radio devices obligates teams to close in on the cryptic sites through other investigative means. In order to effectively estimate possible locations of CRB breeding site, visual monitoring of damage and trapping should assess the presence of CRB populations. Once visual monitoring and trapping indicates the existence of CRB in a particular location, the detector CRB would be released in the vicinity to pinpoint the exact location of the breeding sites. Stats about monitoring and visual in Guam and HI This combination of monitoring methods would ease the control and eradication of CRB, and since traps and visual monitoring are already widespread, it would not be complicated to craft an integrated strategic plan.

**COMPARATIVE TO OTHER METHODS**

**-what did other people use it for/similarities and differences**



**Figure X.**



**Figure Y.** Radio tracked coconut rhinoceros beetles grouped by microhabitat of discovery location. (A) Numbers of beetles tracked to different microhabitats including an indication of percent emergence weight. (B) Percentage emergence weight of beetles (mean ± SE) grouped by microhabitat. Lower case letters represent significant differences (p < 0.05) between beetles found in different microhabitats (Tukey’s HSD). Greek letters represent significant differences between beetles tracked to arboreal microhabitats (> 1 m above ground) or soil-associated microhabitats (< 1 m above ground) (t-test, p < 0.001).

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1. [↑](#endnote-ref-1)