# INVESTIGATING THE EFFICACY OF COMMERCIAL BAITS FOR THE CONTROL OF YELLOW CRAZY ANTS (ANOPLOLEPIS GRACILIPES) AND THEIR IMPACTS ON RED-TAILED TROPICBIRDS (PHAETHON RUBRICAUDA)

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#### **ABSTRACT**

Invasive ants are one of the largest threats to Pacific island ecosystem conservation. I investigated effective ant control options by examining the relative attractiveness of five commercial ant baits to yellow crazy ants (*Anoplolepis gracilipes*). The results were used to select three baits whose efficacy at reducing *A. gracilipes* abundance was then tested in experimental treatment plots. The trials failed to identify an obvious preference for any of the baits and none of experimental treatments resulted in decreases in *A. gracilipes* abundance that differed from untreated plots. Additionally, the impact of *A. gracilipes* on nest initiation rates of Red-tailed Tropicbirds (*Phaethon rubricauda*) was explored. The survey found 90% fewer nest occurred in plots containing *A. gracilipes*. These results demonstrate the negative impacts invasive ants can have on ground-nesting seabirds and suggest that commercial ant baits may be ineffective against controlling *A. gracilipes* supercolonies.

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#### CHAPTER 1

#### THE IMPACTS OF INVASIVE ANTS ON NATURAL SYSTEMS

#### Introduction

Biological invasions can be effective catalysts in precipitating drastic declines to the resilience of native systems and are one of the main drivers of the ongoing decline in global biodiversity (Diamond 1989; Simberloff & Von Holle 1999; Mack et al. 2000; Pimm et al. 2006; Whittaker & Fernandez-Palacios 2007; Brook et al. 2008; Kier et al. 2009). Invasions of isolated island systems tend to be particularly detrimental as these communities are typically characterized as being small, simple, and disharmonic with fewer functional redundancies resulting in correspondingly lower degrees of resilience to perturbations and exacerbated severity of effects generated by disruptions (Elton 1958; Carlquist 1965; Williamson 1981; Simberloff 1995; Usher et al. 1988; Loope & Mueller-Dombois 1989; Wagner & Van Driesche 2010). Augmenting the concern for the fragility of remote island systems is the knowledge that they collectively contain a disproportionally large amount of the world's biodiversity (MacArthur & Wilson 1967; Stattersfield & Capper 2000; Steadman 2006; Kier et al. 2009; Lagabrielle et al. 2009; Illera et al. 2012) and are some of the most threatened natural systems in the world (; Whittaker & Fernandez-Palacios 2007; Kingsford et al. 2009).

Numerous invasive species threaten island systems and their impacts manifest in many forms. Mammalian predators like cats and mongoose can prey upon native species (Hays & Conant 2007; Nogales et al. 2013), invasive mosquitoes can spread disease to previously unexposed populations (van Riper III et al. 1986), and plants like miconia (*Miconia calvescens*) can outcompete and replace native forests with monotypic stands

(Vitousek & Walker 1989; Cronk & Fuller 1995). While predation and the replacement of entire tracts of forest are strikingly apparent examples of invasive species impacts, other less obvious biological invasions can still drive the transformation of entire ecosystems. As inconsequential as they may seem, invasive ants are now recognized as one of the largest threats to Pacific island ecosystem conservation (Nishida & Evenhuis 2000; Wetterer 2002; Steadman 2006; Loope & Krushelnycky 2007; Warwick 2007; New 2008; Rabitsch 2011). Invading ants displace native invertebrate species as well as birds, reptiles, and small mammals through direct predation or competition; they create favorable conditions for successful invasions by other species and have caused the alteration of entire communities (Moller 1996; Holway et al. 2002; O'Dowd et al. 2003; Sanders et al. 2003; Krushelnycky & Gillespie 2008; Kenis et al. 2009; Plentovich 2010). The vast majority of these impacts have been linked to six species of ants: the big-headed ant (Pheidole megacephala), the long-legged or yellow crazy ant (Anoplolepis gracilipes formerly A. longipes), the Argentine ant (Linepthema humile), the red imported fire ant (Solenopsis invicta), the tropical fire ant (Solenopsis geminata), and the little fire ant (Wasmannia auropunctata) (Holway et al. 2002; Loope & Krushelnycky 2007; Rizali et al. 2010; Rabitsch 2011; Roura-Pascual et al. 2011; Wetterer 2011). The ability of these ants to expand their ranges and successfully invade natural ecosystems around the globe, most likely as accidental hitchhikers transported by human commerce, has earned them the designation of "tramp species" (Wilson & Taylor 1967).

The yellow crazy ant is a tramp species of particular concern and has become one of the most wide-spread and damaging in the world (Holway et al. 2002; Wetterer 2005; Chen 2008), leading it to be listed among the top 100 invasive species by the

International Union for Conservation of Nature (Lowe et al. 2004). Though its reputation as a nuisance has been well known for decades (Lewis et al. 1976; Haines & Haines 1978), it did not attain its current level of notoriety until the mid-1990s when extremely high-density supercolonies formed on Christmas Island, a territory of Australia in the Indian Ocean, and dramatically altered the island ecosystem (Green et al. 1999; O'Dowd et al. 2003). This specific incident became a textbook example of an "invasional meltdown" (Groom et al. 2005). Prior to this period, efforts to examine the impacts of invasive ants focused primarily on direct interactions with native ant and arthropod communities, with most observations of impacts on non-invertebrate taxa limited to anecdotal observations (Holway et al. 2002). However, after the invasional meltdown on Christmas Island, efforts broadened to define and explore the impacts that supercolony-forming invasive ant species like *A. gracilipes* can have on non-invertebrate taxa.

Over the past twenty years, scientists and resource managers have come to recognize *A. gracilipes* as a virulent invasive species whose impacts can cascade across multiple trophic levels. Yellow crazy ants have been documented to cause the death of newly hatched chickens and newly born domestic animals such as pigs, dogs, cats, and rabbits (Haine et al. 1994), out-compete and depredate other native ants and invertebrate fauna (Gillespie & Reimer 1993; Hill et al. 2003; Lester & Tavite 2004; Abbott et al. 2007; Bos et al. 2008; Mezger & Pfeiffer 2011), extirpate land crabs and reptiles (Haines & Haines 1978; Feare 1999; McNatty et al. 2009), predate chicks and reduce nesting success of seabirds (Feare 1999; Plentovich et al. 2011), disrupt foraging and breeding of land birds (Davis et al. 2008, 2010; Matsui et al. 2009), play a role in canopy die-back and tree death (Hill et al. 2003; O'Dowd et al. 2003), contribute to cascading effects on

entire biotic communities (Savage et al. 2009, 2011; Savage & Whitney 2011), and precipitate catastrophic change to an entire rainforest ecosystem (O'Dowd et al. 2003). While Gruber et al. (2013) recently described several populations of *A. gracilipes* on Tokelau atolls declining on their own to levels that no longer cause detectable ecological damage, the majority of studies on invading ants species have found increased abundance and intensified impacts on native communities over time (O'Dowd et al. 2003; Simberloff & Gibbons 2004; Hoffmann & Parr 2008). The extent and severity of these impacts justify the description of *A. gracilipes* as one of the six "most widespread, abundant, and damaging of invasive ants" (Holway et al. 2002).

#### **Invasive Ant Impacts on Seabirds**

The breeding biology of ground-nesting seabirds makes them particularly susceptible to negative impacts from invasive ants. High-density breeding colonies provide ample resources for the growth of ant populations in the form of guano, boluses, eggs, chicks, and dead adults. Most seabird species have part-time pair bonds, meaning they do not spend the non-breeding season together and their previous nest site acts as a meeting point in order to reunite each breeding season (Bried & Jouventin 2002). If a breeding pair is displaced from their meeting site, the birds may fail to reunite and could miss one or more breeding seasons, which can lead to reduced lifetime reproductive success and possibly localized population declines (Bried et al. 2003). In addition, nest-bound chicks and brooding adults are susceptible to physical harm from invading ants for the simple reason that they need to remain at the nest site. Young chicks are physically incapable of moving, and abandonment by adults will result in the failure of the nest.

Even if ants do not directly depredate nest-bound birds, harassment from swarming ants spraying irritating formic acid can induce constant preening resulting in increased energy expenditure, which could lead to reduced nesting success (Vinson 1994). The intrinsically low rates of population growth that characterize seabird populations leave them especially vulnerable as even small declines in breeding success can have long-term consequences (Hamer et al. 2002).

Currently, there are only seven published accounts that describe interactions between invasive ants and seabirds (Lockley 1995; Feare 1999; Krushelnycky et al. 2001; McClelland & Jones 2008; Plentovich et al. 2009, 2011; DeFisher & Bonter 2013). Lockley (1995) found that hatchling mortality was higher in Least Terns (Sternula antillarum formerly Sterna antillarum) in areas where red imported fire ants (S. invicta) were not controlled compared to areas treated with pesticide. Feare (1999) documented the displacement of an estimated 60,000 nesting Sooty Terns (Onychoprion fuscatus formerly Sterna fuscatus or S. fruscata) and the deaths of White Tern (Gygis alba) chicks that were attributed to an invasion of A. gracilipes. McClelland & Jones (2008) attributed the failure of a Tristram's Storm-petrel (Oceanodroma tristrami) nest to swarming pharaoh ants (*Monomorium pharaonis*) that forced the chick from its nest area. Plentovich et al. (2009) found an increase in Wedge-tailed Shearwater (Puffinus pacificus) fledging success on an islet where the previously dominant tropical fire ant (S. geminata) was controlled relative to an adjacent islet where ants were not controlled. In the same study, no difference was found in fledging success on similarly treated islets that were dominated by big-headed ants (*Pheidole megacephala*). However, one islet was invaded by A. gracilipes following eradication of P. megacephala and there was a

corresponding reduction in seabird nesting success (Plentovich et al. 2011).

Krushelnycky et al. (2001) found no impact of Argentine ants (*Linepthema humile*) on Hawaiian Petrels (*Pterodroma sandwichensis*) though they were unable to make any direct observations of petrel chicks due to the physical constraints of monitoring nests in burrows. DeFisher and Bonter (2013) found that Herring Gull (*Larus argentatus*) chick growth rates were lower in nests with European fire ant (*Myrmica rubra*) activity.

Overall, invasive ant impacts on seabirds remain understudied. These studies in combination with fairly widespread anecdotal observations indicate that invasive ants can have devastating impacts on nesting seabirds.

Given the number of problems that have been attributed to invasions of *A*. *gracilipes* and the additional threats seabird species face to their survivorship (Nettleship et al. 1994; Boersma et al. 2002, Hatfield et al. 2012), Chen's (2008) findings that future climate scenario models predict a substantial global expansion of *A. gracilipes* distribution is a cause for concern. Increasing management actions will be necessary to conserve native biodiversity as *A. gracilipes* and other invasive ants spread to new areas, and such action will require effective and efficient tools.

#### **Invasive Ant Management in Conservation Areas**

Ants are highly successful colonizers (McGlynn 1999) and have proven especially difficult to control and eradicate once established (Holway et al. 2002). Individuals in a single colony can number from hundreds to millions, and those visible on the surface are typically only a small fraction of the entire colony (Holldobler & Wilson 1990). If even a few workers escape with a queen or eggs from which a queen can be raised, eradication

efforts are likely to fail. In addition, ant species vary considerably in their foraging strategies, social structures, and life cycles, and these in turn can vary seasonally within each species (Holldobler & Wilson 1990). This could explain why ant eradications are notoriously difficult and why no single ant pest management strategy has been shown to be consistently effective. Though challenges remain, the feasibility of ant eradication efforts has been demonstrated by a number of modest successes (Reimer & Beardsley 1990; Abedrabbo 1994; Hoffmann & O'Connor 2004; Causton et al. 2005; Hoffmann 2010; Plentovich et al. 2011).

It is generally accepted that the most effective and efficient method of ant control is the broadcast of chemical baits (Williams et al. 2001; Stanley 2004; Krushelnycky et al. 2005). This strategy relies on a common trait among ant species called trophallaxis, which is the sharing of regurgitated food among colony members (Holldobler & Wilson 1990). The concept behind bait control and eradication strategies assumes that foragers will consume or retrieve food items they find in the environment and return to the colony where the food is shared with other colony members. By incorporating an insecticide into an attractive food item, the foragers themselves become the mechanism by which the insecticide is delivered to queens, larvae, and workers that do not leave the nest and would not otherwise come into contact with the chemical. Bait applications have been documented to be a reliable method for the control of many ant species though the type of attractants and chemicals employed has varied by situation (Knight & Rust 1991; Forschler & Evans 1994; Vail et al. 1996; Lee 2000, 2007; Lee et al. 2003; Krushelnycky et al. 2011; Gaigher et al. 2012).

While a baiting strategy is a seemingly simple concept, an effective bait must incorporate species- and environment-specific considerations which are especially important if the goal is eradication. These include ensuring an adequate rate of bait uptake, the effectiveness of the formicide once ingested, and the financial and potential ecological non-target costs of the baiting strategy (Stringer et al. 1964; Rust et al. 2004).

Identifying a preferred food source that foraging ants readily consume is the first crucial step in developing a bait. The ants must be sufficiently attracted to the bait that they readily take it up in quantities sufficient to ensure delivery to non-foraging members of the colony before the toxicant kills the original foragers or environmental conditions degrade the bait (Davis & Van Schagen 1993; Lee 2000; Stanley 2004; Stanley & Robinson 2007). The bait must be not only attractive, but also come in a form that can be mixed with an insecticide and shared among ants via trophallaxis.

Selecting an appropriate specific chemical control agent and determining its most effective concentration is also a challenge that requires a very fine balance. It is critical that the formicide mixed into the bait exhibit delayed toxicity, in order to avoid the death of foraging workers before they can return to the nest with the bait (O'Dowd et al. 1999; Rust et al. 2004). The ideal period of delay would be of a sufficient duration to allow for individual foragers to make multiple trips and recruit other workers to the bait. The toxicant that is selected must not only avoid killing the foraging worker too quickly, but must exhibit toxicity over a range of doses since it will progressively dilute as it is shared between ants and spread through the colony over time (Markin 1970). In addition, the toxicant must not reduce the bait's attractiveness to the foragers. If any of these criteria fail to be met, the formicide is unlikely to reach all members of the colony, and though

some degree of suppression may be achieved, eradication would be less likely (Stringer et al. 1964).

Even when a bait treatment is identified, its use must be vetted against considerations beyond simply the scope of its effectiveness at killing ant colonies. Minimizing direct and indirect negative impacts that the control efforts have on native communities is paramount. These impacts can range from the fairly predictable deaths of other invertebrates that consume the bait (Stanely 2004; Green & O'Dowd 2009; Plentovich et al. 2010) to unexpected shifts in the local biotic community after a target population has been removed (Plentovich et al. 2011). Adjustments to the baiting strategy may need to be considered if the risk to desirable native species outweighs the threat the target organism represents. As with all management activities, the preferred strategy must also pass economic and regulatory standards. Usage of toxicants that offer promising results may not comply with local regulations, and strategies that effectively remove pests from an average-sized household may be too costly or impractical to apply at a landscape scale.

Once a bait is selected, the next step in formulating the baiting strategy is to determine how to deliver the bait to the target organism. Common ant control methods include the broadcast application of chemical baits and the deployment of bait stations (Reimer & Beardsley 1990; Krushelnycky 2008; Green & O'Dowd 2009). The use of bait stations has some advantages over broadcast applications because the stations can shield the bait from environmental degradation, thereby increasing the amount of time it is available to the target organism, and the stations can be designed to exclude non-target organisms from coming into direct contact with the bait. Unfortunately bait stations are

often financially and logistically infeasible when dealing with large scale infestations or those occurring in less accessible areas, which may explain why the majority of successful ant control and eradication projects have involved broadcast methodologies (Hoffmann & O'Conner 2004; Plentovich et al. 2009; Boland et al. 2011; Peck et al. 2013).

When considering the products available for ant control, it is important to appreciate the limitations of relying on commercially purchased baits. Commercially available ant control products target species for which there is a sufficient market demand to generate a profit for the manufacturer, and focus on control rather than eradication (Williams et al. 2001; Stanley 2004). For instance, an American-based company is unlikely to spend resources researching, producing, and obtaining regulatory approval to market a bait targeting jack jumper ants (Myrmecia pilosula), a species not known to occur in America. If *M. pilosula*, or any other new species, were to be suddenly discovered imposing detrimental impacts to a valued conservation area, it would be unlikely that any commercial bait would meet all the criteria needed to achieve a successful eradication. With at least 150 species of ants currently undergoing humanmediated range expansions (McGlynn 1999; Holway et al. 2002; Chen 2008), species are regularly introduced into new environments and new countries, and they may differ just enough in their foraging strategies to render approved baits designed for known species ineffective. Whenever novel combinations of species and environments occur, baits should be thoroughly field tested prior to investing the limited resources that managers have at their disposal.

The discovery of an invasion of *A. gracilipes* at Johnston Atoll National Wildlife Refuge in 2010 epitomizes a situation in which a newly introduced species was causing detrimental environmental impacts, but no species-specific control products were available. While *A. gracilipes* is recognized as a significant pest species around the world (see above), no previous cases of substantial impacts in the United States have been documented save for several conservation and agricultural areas in Hawai'i (Gillespie & Reimer 1993; Plentovich et al. 2011; Nelson & Taniguchi 2012). These impacts alone have not generated a sustainable consumer demand, and no commercial baits specifically formulated to target *A. gracilipes* have been made available in American markets. Facing the potential loss of critical seabird habitat at Johnston Atoll (Flint & Woodward 2010), resource managers identified a need to develop an *A. gracilipes* eradication program, the first step of which was to examine the efficacy of available commercial ant baits.

#### **Study Site and History of Johnston Atoll**

Johnston Atoll is an unincorporated U.S. territory and has the distinction of being one of the most remote atolls in the world (Amerson & Shelton 1976). Located in the central Pacific Ocean, it is approximately 1,300 km west-southwest of Honolulu, Hawaii at 16.74°N, 169.52°W. The atoll is composed of a shallow coral reef complex situated atop an isolated seamount platform that is approximately 33.8 km in circumference. Johnston originally contained two small islands, Johnston (24.3 ha) and Sand (5.3 ha), which have since been expanded to their current sizes of 253 ha and 9 ha, respectively, in order to fulfill United States military mission needs. In addition, the military built two

completely man-made islands, the 10.1-ha North (Akau) Island and 7.3-ha East (Hikina) Island. With the exception of the naturally occurring east lobe of Sand Island, all terrestrial habitat at Johnston Atoll is essentially man-made and consists of compact coral substrate harvested by dredging of the surrounding coral reefs.

After several periods of exploitation by guano minors and feather collectors,
Johnston and Sand Islands were designated as a reserve for breeding birds in 1926. The
protective status was eventually extended to include the surrounding waters in what is
now known as Johnston Atoll National Wildlife Refuge (NWR). However, in 1934 the
jurisdiction of Johnston Atoll was turned over to the U.S. Navy as result of its strategic
location and increasing political hostilities that would eventually lead to World War II.
In 1939, construction of the first permanent military facilities began on the atoll. For the
following 65 years, Johnston hosted active U.S. military operations until its final closure
and abandonment in 2004 (USAF 2004). In 2009, a Presidential Proclamation
established the Pacific Remote Islands Marine National Monument which encompasses
Johnston Atoll and its surrounding waters out to 50 nautical miles and provides the atoll
with additional political stature and protections.

Despite more than 6 decades of intense human activity and disturbance that has completely altered the terrestrial habitat, 15 seabird species continue to maintain colonies at Johnston Atoll. As the only emergent land in approximately 850,000 square miles of ocean, Johnston provides a significant and critical breeding habitat for these species. At the time of base closure in 2004, there was great optimism for the potential the atoll held to host increasing numbers of breeding seabirds. It was reasoned that by simply halting all disturbances that resulted from prior military operations, seabird numbers would

increase over time on their own with minimal further investment of management resources. Between 2004 and 2010, Johnston Atoll remained uninhabited and atoll-based management activities were primarily limited to brief occasional monitoring visits by refuge biologists roughly once every two years. While these visits were inadequate to document clearly any increases in specific seabird populations, biologists were able to document the return of five breeding seabird species to Johnston Island whose nesting had been previously constrained to the smaller outer islands (Woodward & Hayes 2009). The reoccupation of Johnston Island by these birds suggested the birds were responding as predicted to the absence of disturbance.

Though unpermitted entry into the atoll is prohibited, the isolated location makes enforcement impractical. As a result, the biologists who occasional visit the atoll have reported multiple signs of trespass since 2004 (USFWS, unpublished data). While the exact means by which *A. gracilipes* arrived at the atoll may never be known, they may have been unintentionally introduced by a trespassing vessel between 2008 and 2010.

#### **Yellow Crazy Ants on Johnston Atoll**

A. gracilipes was first documented on Johnston Atoll in January 2010 when visiting USFWS biologists observed ants swarming in high densities over an estimated 50-ha area (Fig. 1.1) (Flint & Woodward 2010). It was reported that the ants appeared to have dramatic negative effects on ground-nesting seabirds, which were exhibiting signs of extreme agitation and duress as they continuously tried to rid themselves of swarming ants. Observers reported Red-tailed Tropicbirds (*Phaethon rubricauda*) that appeared to be blinded by the formic acid the ants spray. The overall result of this harassment was

the apparent abandonment of the invaded area by almost all ground-nesting birds. The threat of the ants spreading across the entire 253-ha island and completely displacing multiple species of seabirds became a critical concern for resource managers.

### Johnston Atoll NWR, Crazy Ant Infestation and Treatment Areas

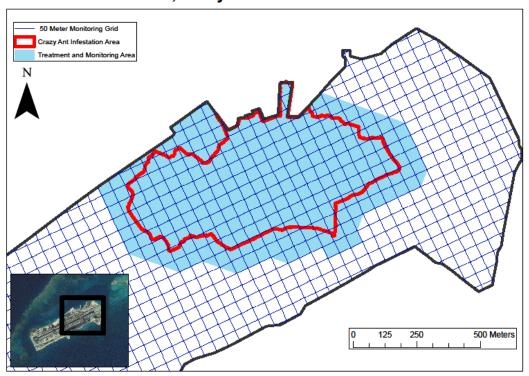


Figure 1.1. Map of Johnston Island indicating the January 2010 estimated extent of the yellow crazy ant (*A. gracilipes*) infestation (red outline) and proposed treatment area (blue polygon). The 50-m grid was deployed across the entire island and used as a framework for monitoring and management activities.

Here, I report on investigations aimed at identifying a suitable and effective bait treatment to control invasive population of *A. gracilipes* on Johnston Atoll NWR and explore the population's impact on nesting numbers of *P. rubricauda*. In order to identify the ant baits for which *A. gracilipes* shows the highest preference, I examined the relative attractiveness of five commercial ant bait products using standardized trials. I then used the results of those trials to select three baits and examined their efficacy in

reducing *A. gracilipes* forager abundance in field trials using experimental treatment plots. Surveys for *P. rubricauda* were conducted in two sets of sample plots to examine the difference in the number of nests in invaded versus uninvaded areas to determine if nest numbers were affected by the presence of *A. gracilipes*. These efforts were undertaken to test the following hypotheses.

- 1) A. gracilipes will show varying visitation rates to each of the commercial ant baits.
- 2) The number of foraging *A. gracilipes* will decline when the selected baits are applied in the field according to label restrictions in experimental versus control plots.
- 3) The relative efficacy of each type of bait treatment in reducing the number of foraging *A. gracilipes* will vary between the different treatments.
- 4) The number of *P. rubricauda* nest initiations will be lower in areas where *A. gracilipes* is present relative to uninvaded areas.

#### **CHAPTER 2**

# RELATIVE ATTRACTIVENESS OF FIVE COMMERCIAL ANT BAITS TO ANOPLOLEPIS GRACILIPES (HYMENOPTERA: FORMICIDAE)

#### Introduction

Invasive ant species are a major threat to the conservation of biodiversity on oceanic islands (Nashida & Evenhuis 2000; Wetterer 2002; Loope & Krushelnycky 2007; New 2008; Plentovich 2010; Rabitsch 2011). In the face of the current human-mediated range expansions of close to 150 ant species (McGlynn 1999; Holway et al. 2002; Wetterer 2005), resource managers are responding to an increasing number of ant invasions into areas of high conservation value (Kolar & Lodge 2001; Lester 2005). The need for effective and efficient ant control tools is intensifying. Historically, research on controlling pest ants over large areas has focused on high-toxicity pesticide sprays (Williams 1993). While this technique was often adequate to control ant colonies by knocking down their numbers, it often failed to eradicate the colonies and at times introduced chemicals into the environment at levels detrimental to non-target organisms (Carson 1962). While the use of many of the most environmentally harmful pesticide sprays has been discontinued, current commercially available ant control products are often unsuitable for use on invasions in conservation areas because they are ineffective on the target species, have not been registered for such applications, or risk to non-target native organisms precludes their use (Krushelnycky et al. 2005; Rabitsch 2011).

In the last twenty years, research into ant control efforts has begun to focus on the use of chemical control agents formulated into attractive bait matrices (Williams 1993; Stanley 2004; Krushelnycky 2005; Plentovich et al. 2010, 2011; Stewart et al. 2014,

2014). This technique takes advantage of trophallaxis, which is the sharing of regurgitated food among colony members (Holldobler & Wilson 1990). The concept behind bait control and eradication strategies assumes that foragers will consume or retrieve food items they find in the environment and return to the colony where the food is shared with other colony members. By incorporating a chemical control agent into an attractive food item, the foragers themselves become the mechanism by which the insecticide is delivered to queens, larvae, and workers that do not leave the nest and would otherwise not come into contact with the chemical. While the complete removal of established ant populations has proven difficult (Holway et al. 2002), bait applications have been demonstrated to be a reliable method for the control of many ant species (Knight & Rust 1991; Forschler & Evans 1994; Vail et al. 1996; Lee 2000; Lee et al. 2003; Lee 2007; Krushelnycky et al. 2011; Gaigher et al. 2012) and have been used in the few documented ant eradications that have been successful in natural areas (Reimer & Beardsley 1990; Abedrabbo 1994; Hoffmann & O'Connor 2004; Causton et al. 2005; Hoffmann 2010; Plentovich et al. 2011).

In areas of conservation value, bait applications have additional attributes that make them preferable over traditional contact pesticide sprays. Bait that is consumed requires relatively small amounts of active ingredients, reducing the amount of chemicals in the environment. In addition, a bait matrix can be tailored to both appeal to the target species and reduce the likelihood of contact with non-target organisms (Williams 1993). A bait strategy incorporating the use of recently developed reduced-risk synthetic insecticides (e.g., pyrethroids, neonicotinoids, and insect growth regulators) offers promising options to conservation managers facing a Christmas Island-like scenario in

which an ant invasion threatens catastrophic impacts (Green et al. 1999; O'Dowd et al. 2003). However, given that the majority of commercial bait products target only a few species, field trials are required to assess the efficacy of these baits on other species prior to large scale treatments and managers may be forced to develop specific baits tailored for each situation (Stanley 2004; Gentz 2009).

There are a number of vital aspects in designing a bait control program that can influence its efficacy, and each aspect can differ between species, environments, and seasons (Stein 1990; Stanley 2004). While factors such as the method of bait delivery, preference for other food resources in the environment, non-target consumption of the bait, and the foraging strategy of the target species must be taken into account, they are often ancillary to the development of the chemical bait itself (Stanley 2004; Krushelnycky et al. 2005). In order for a bait to be successful at eradicating an ant population, it is critical that it meet three criteria: the bait must be attractive to the target species; it must be able to be shared between members of the colony; and the ants must take it up at a sufficiently high rate to ensure it is shared with other colony members before the effect is felt by the original foragers (Davis & Van Schagen 1993; Williams 1993; Lee 2000; Rust et al. 2004; Stanley & Robinson 2007). The development of chemical baits poses many challenges, but efficacy is ultimately determined by two actions: the uptake of the bait by the ants and the efficacy of the toxin (Williams 1993). While the chemical agents often get the lion's share of the credit for delivering the final coup de grâce, the underappreciated bait matrix exerts greater influence over the success or failure of an eradication effort. The overall attractiveness of the bait matrix determines whether a sufficient amount of the chemical control agent is delivered to achieve a successful eradication.

Bait matrices have four physical components: attractants (foods or pheromones), carriers (physical structure), toxicants, and additives (e.g., preservatives, dyes, etc.) (Klotz et al. 1997). While toxicants and additives have important functions, they should have a neutral effect on the rate of uptake of the bait and as a rule should not repel ants when mixed into the matrix. The attractant and carrier determine the relative attractiveness of baits and the rate of uptake into the colony. The choice of attractant will depend on preferences exhibited by the target organism which often involve a type of food (e.g., carbohydrates, lipids, or proteins), while the preferred carrier can differ in form or consistency (e.g., granule, liquid, or gel) (Williams 1993). When a final bait product is dispersed in a natural setting, it will be in competition for each forager's attention with all the other resources available in the environment. If the bait is not optimized to engender a rapid and substantial uptake response, it may not spread through the entire colony before the foragers succumb to the toxin or the bait degrades.

While high selectivity and low non-target impacts heighten the appeal of baits tailored to specific ant species, those same traits can result in products that are highly effective on only one or a few ant species (Gentz 2009). The bulk of research on ant control methodologies has focused on just a few species, such as the red imported fire ant (*Solenopsis invicta*) (Williams 1993; Stanley & Robinson 2007), leaving the majority of invasive ant species understudied with respect to both ecology and control methodologies (Holway et al. 2002). This disparity is reflected in the few common pest

species that commercial ant baits have been developed to address (Stanley 2004), leaving the efficacy of those baits untested on the majority of ant species.

The yellow crazy ant (*Anoplolepis gracilipes*) is one of the world's most widespread, abundant, and damaging invasive ants (Holway et al. 2002). They are capable of invading natural environments and attaining extremely high densities that impose detrimental impacts to native insects (Gillespie & Reimer 1993; Hill et al. 2003; Lester & Tavite 2004), crustaceans (Green et al. 1999; McNatty 2009), reptiles (Haines et al. 1994), forest birds (Davis et al. 2008, 2010; Matsui et al. 2009), seabirds (Feare 1999; Plentovich et al. 2011), and trees canopies (O'Dowd et al. 2003; Hill et al. 2003), which contribute to cascading effects on entire communities (Savage et al. 2009, 2011; Savage & Whitney 2011), and precipitate catastrophic change to an entire rainforest ecosystem (O'Dowd et al. 2003). The documented loss of biodiversity and ecosystem integrity following *A. gracilipes* invasions coupled with the species' historic and global potential range expansion (Wetterer 2005; Chen 2008) make it a high risk species (Stanley 2004) and justify its inclusion as one of the top 100 invasive species in the world (Lowe et al. 2004).

Here, I report on an investigation into the attractiveness of five commercial ant bait products on a recently established colony of *A. gracilipes* on Johnston Atoll National Wildlife Refuge in the central Pacific Ocean. The purpose of this trial was to examine the relative attractiveness of commercial baits in order to identify which, if any, receive the most visits by *A. gracilipes* by testing the hypothesis that *A. gracilipes* will show varying visitation rates to each of the commercial ant baits.

#### Methods

Study Site

Johnston Island is located within Johnston Atoll National Wildlife Refuge, an uninhabited, unincorporated United States territory. The atoll, located at 16.74°N, 169.52°W, has the distinction of being one of the most remote in the world. Though natural in origin, the island was artificially expanded to its current size of 253 ha and was the site of an active military base for nearly 75 years until its closure and abandonment in 2004. Not surprisingly, the current terrestrial environment is composed primarily of introduced plants and invertebrates and a few those plants and insects that have been able to survive since the abandonment of the previously intensively managed anthropogenic landscape (Amerson & Shelton 1976). As Johnston Atoll contains the only emergent land in approximately 2 million square kilometers of ocean, the terrestrial environment also provides significant and critical habitat for 15 species of breeding seabirds.

In 2010, a supercolony of *A. gracilipes* was discovered spanning a continuous 50-ha area of the island. From within this area, three sites containing four subplots each were selected that represented three common habitat types that occur patchily across the island. Site 1 subplots were located on loose sandy soil covered with vegetative litter and under tree canopies over 5 m in height that provided 50-75% canopy cover directly over the subplots. Site 2 subplots were located in separate open patches of bare ground composed of coarse coral substrate and were each surrounded almost exclusively by 0.5-1.5 m tall *Pluchia indica* shrubs that provided 50-75% cover over the surrounding area. Site 3 was located in an open area of sparse mixed herbs and grasses with all vegetation under 0.5 m on a fine sandy soil substrate. The 3 sites were between 180 and 250-m

apart and all subplots within each site were at least 5 m apart but no more than 30-m apart.

Trials were conducted with four subplot replicates at each of the three sites. Each subplot consisted of 6 permanently marked stations arranged at 1 m intervals around the perimeter of a circle for a total of 24 stations at each of the 3 sites. Single-use monitoring pads were placed at each station about five minutes prior to the beginning of each trial. Monitoring pads consisted of a 6.5 cm diameter circular piece of reinforced cellulose fabric (Scott shop towels) with a metal hardware washer (2.5 cm outer diameter, 1.1 cm inner diameter) glued to the center of the bottom side. On the upper side of the monitoring pad, the inner and outer edges of the washer were outlined in ink. The inner circle circumscribed the shallow bowl caused by the hole in the washer and defined the bait placement area. The space between the outer and inner circles defined the count area in which any *A. gracilipes* entering was counted.

#### Data Collection

Four observers conducted bait attractiveness trials on six days between 3 February and 3 March 2011. On each day, trials were repeated at each of the three sites in three separate sessions that occurred at first light, at midday, and two hours before sunset for a total of nine trials per day. All trials conducted at least 2 days apart when wind was below 15 knots and no rain had fallen within 24 hours. Immediately prior to the start of each trial, observers placed one monitoring pad at each of the six marked stations in each subplot and then positioned themselves on a stool in the center of the circle. Trials began at all four subplots within a site simultaneously as each observer placed the first of five test baits on the first station. Baits were applied in equal volumes to the center of

each monitoring pad to completely cover the approximately 1 cm<sup>2</sup> bait well. Observers recorded the number of *A. gracilipes* that entered the count area as the ant approached the bait over a two-minute period. If an ant entered the circle it was counted regardless of whether the same ant had left the circle and was seen returning multiple times. At the end of two minutes observers recorded a snapshot count of the total number of ants in the circle at that moment. The monitoring pads were left in place, and an additional snapshot count was conducted 60 minutes after the start of each trial. In each trial, one monitoring pad was left empty as a control and five commercial ant baits were tested (Table 2.1).

Table 2.1. Commercial ant baits used in field trials to determine relative attractiveness to *A. gracilipes*.

Commercial bait by matrix form	Active ingredient (concentration)	Manufacturer
Gel		
Combat Source Kill Max A2 Ant Killing Gel	fipronil (0.01%)	The Dial Company, USA
Liquid		
Maxforce Quantum Ant Bait	imidacloprid (0.03%)	Bayer Environmental Sciences, USA
Granular		
Maxforce Complete Brand Granular Insect Bait	hydramethylnon (1.0%)	Bayer Environmental Sciences, USA
Maxforce Fine Granular Insect	hydramethylnon (1.0%)	Bayer Environmental
Bait Amdro Granular Insecticide	hydramethylnon (0.73%)	Sciences, USA Ambrands, USA

After completing the 2-minute count periods at all 6 stations at a subplot, the observers moved to the second site and repeated the process and then again at the third sight before returning to the first site to complete the 60-minute counts. To account for potential bias if one station was closer to an area with higher ant activity (e.g., nest

entrance or foraging trail), the placement of the baits were rotated clockwise by one station each day so that each bait was trialed once at every station.

#### Data Analysis

I used the mean number of ant visits during the first 2 minutes and the mean number of ants present at 2 and 60 minutes at bait stations as indicators of *A. gracilipes* bait preference. Session means for each site were pooled each day and the means of the 2-minute and 60-minute snapshot counts were averaged. One-way Analysis of Variance (ANOVA) and Tukey-Kramer post-hoc tests were used to determine if there was a difference in the mean ant visits and ant presence among the six treatment groups (Minitab 16 Statistical Software 2010). The analysis was performed on a 2-minute visitation measurement and a measurement of the average number of ants presents at one time, each of which was log-transformed to improve normality and resulted in sample sizes of 18 observations for each measurement for each of the 6 treatments.

#### Results

Trials with four replicates for each of the six treatments (five baits and one control) were completed at three sites, three times a day for six days. A total of 216 observations of each of the 3 measurement variables for each of the 6 treatments were made. *A. gracilipes* was the only ant observed during all trials with the exception of one occurrence of a single *Ochetellus glaber* observed at an Amdro Granular Insecticide (Amdro) bait station. A total of 16,381 *A. gracilipes* visits were counted in the 1,296 individual 2-minute count periods during the study. Midday observations were excluded from the analysis due to consistently low ant activity levels that resulted in 73% of all

pooled midday observations detecting no ant visits. The remaining 864 observations had an average of 14.5 *A. gracilipes* per station in the first 2 minutes with 11% receiving no ant visits and a maximum count of 114 visits at a single station.

There was a significant difference in mean number of A. gracilipes visits in the first two minutes across the six treatments (F=4.594, df=5, p=0.001). The Tukey-Kramer MSD identified Combat Source Kill Max A2 Ant Killing Gel (Combat), Maxforce Fine Granular Insect Bait (Fine Granular), and Maxforce Quantum Ant Bait (Quantum) as differing significantly from the control but not from each other or from Amdro or Maxforce Complete Brand Granular Insect Bait (Complete) which did not significantly differ from the empty control pad (Fig. 2.1).

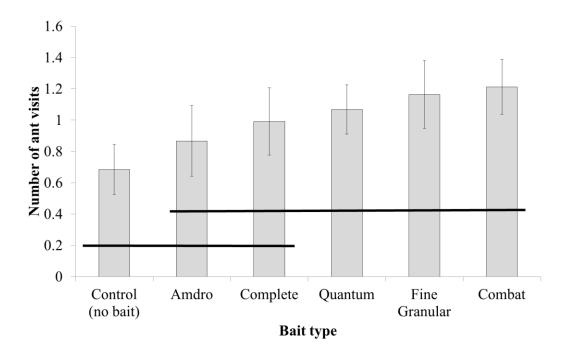


Figure 2.1. Comparison of the mean number of A. gracilipes visits to five commercial baits and an empty control pad over a two-minute period. Treatments that are connected by a horizontal black line are not significantly different from each other (p>0.05). Bars = 95% confidence interval, values are log transformed, n=18 for each treatment.

The means of the number of ants present during the snapshot counts were significantly different between the six treatments (F=19.821, df=5, p<0.001). The Tukey-Kramer MSD revealed that Combat and Quantum had significantly more ants present than the other four treatments. Amdro, Complete and Fine Granular did not significantly differ from the control or from each other (Fig 2.2).

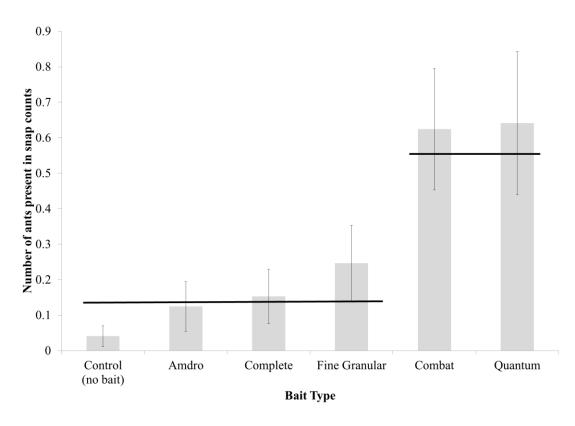


Figure 2.2. Comparison of the mean number of *A. gracilipes* present during counts at 5 commercial baits and an empty control pad 2 minutes and 60 minutes after the bait was presented. Treatments that are connected by a horizontal black line are not significantly different from each other (p>0.05). Bars = 95% confidence interval, values are log transformed, n=18 for each treatment.

#### **Discussion**

Developing an effective *A. gracilipes* control strategy is a complicated process with many critical components that all must meet certain criteria to ensure the highest

likelihood of success. Identifying a bait matrix that is suitably attractive to *A. gracilipes* is just one among several steps to be addressed. To proceed in the most cost effective and efficient way, we identified and tested readily available commercial baits in order to determine which showed the most promise and could be recommended for further efficacy testing.

The bait attractiveness test results revealed that Combat, Fine Granule, and Quantum attracted significantly more ants relative to the control pad. However, none of the five baits differed significantly from each other in the number of *A. gracilipes* visits in the first two minutes of bait presentation. Quantum and Combat did have significantly more ants present then Fine Granular during snapshot counts at 2 and 60minutes, but they did not differ from each other. While some differentiation can be deduced from these results, overall they suggest that *A. gracilipes* does not strongly favor any of the tested baits.

Although Amdro had double and Complete had triple the total number of ant visits in two minutes than the empty control pad, the difference was not significant in either analysis. While *A. gracilipes* may be moderately attracted to these two baits they are unlikely to induce a strong enough recruitment response to ensure adequate uptake into the colony. In dry environments these granular baits may persist long enough for a substantial number of ants to eventually feed on them but in areas with even moderate rainfall or humidity the dry granules will likely become unattractive within hours to days (SJK personal observation). Both analyses are in agreement that neither Amdro nor Combat can be recommended to control *A. gracilipes*.

Fine Granular was the only granular bait to attract significantly more ants then the empty control pad and received the second greatest number of visitations in the first twominute period. However, it did not differ significantly from the empty control pad in the snapshot counts. When considering the results of the 2- and 60-minute snapshot counts it is important to consider the potential bias that may exist in the experimental design when comparing granule and liquid baits. Counts of the number of ants present at bait in a single moment may be affected by the amount of time it takes for an ant to physically remove the bait. An ant may readily pick up and carry away a granule within a few seconds compared to liquids and gels that require the ant to consume the bait and remain in place longer. A much greater number of ants could potentially visit a granular bait station than could visit a liquid bait station, but a snapshot count comparison may falsely indicate the liquid bait is more attractive because the lower number of individuals remained at the bait for a longer period and all remained present during the snapshot count. We saw examples of this during the trials, when on several occasions we returned for the 60-minute counts to find most of the Fine Granule bait gone, suggesting it was very attractive, but only a few ants happened to be at the remaining bait during the count. Due to this bias, it is likely that the 2- and 60-minute snapshot counts of ants present underestimate the attractiveness of granular baits when they are compared to liquid baits. By counting the total number of ant visits over a period of time, the two-minute visitation rankings are not subjected to this bias and are more likely to represent an accurate picture of the relative attractiveness of the baits regardless of their form. While I do not completely disregard the snapshot count data, the results of the two-minute cumulative

visitation test show that the Fine Granule bait had the second-highest number of visiting ants with only Combat receiving more visits.

Quantum was attractive to A. gracilipes and ranked progressively higher with each additional measurement. While quantum ranked a distant third in mean number of ant visits over 2 minutes, it ranked second above Fine Granule in 2-minute snapshots and surpassed the second place Combat by 50% in 60-minute snapshot counts. Whereas the potential bias in snap shot counts may underestimate the attractiveness of granular baits, it may overestimate the attractiveness of liquid and gel baits. Quantum is a syrupy liquid that ants are unable to carry off in large quantities unless it is consumed. Compared to the time it takes to pick up a piece of granular bait, it will likely require a longer period of time to consume a viscous liquid. This translates into longer period of visitations by ants at liquid baits. When ant abundance at a bait at a given moment in time is used as a proxy for measuring relative attractiveness, a bias could be introduced if the baits take different forms. In the case of this study it may by prudent to consider comparing only baits that have similar physical forms in the snap shot analysis. In the case of Quantum and Combat, they did not significantly differ in number of ants present in the snapshot analysis. Quantum attracted significantly more ants than the empty control pad in both analyses though it did not differ significantly from any of the other baits in the twominute count or from the only other non-granular bait in the snap shot count. While I can confidently state that Quantum is more attractive to A. gracilipes than Amdro and Complete, its ranking in relation to Fine Granular and Combat has not been clarified by the results of this study. Overall, Quantum's results justify further consideration and

testing of its efficacy against *A. gracilipes* though it did not clearly standout as the preferred option.

Combat was clearly the most attractive of the baits tested on *A. gracilipes*. It received the highest average number of ant visits in the first 2 minutes among all five baits tested. When compared with Quantum in the snap counts it had more ants present at the 2-minute snapshot count and slightly fewer ants present at 60-minute snapshot count though when averaged Quantum had slightly more ants present. Combat and Quantum were the only two baits to attract significantly more ants than the empty control pad in both analyses. However, as with Quantum the potential bias mentioned previously should be considered when formulating a conclusion.

While failing to identify an unqualified option, the bait attractiveness results presented here support further study to assess the efficacy of Combat, Quantum, and Fine Granule baits in the control of *A. gracilipes*.

#### **CHAPTER 3**

# FIELD EVALUATION OF THE EFFICACY OF THREE COMMERCIAL ANT BAITS FOR THE CONTROL OF ANOPLOLEPIS GRACILIPES (HYMENOPTERA: FORMICIDAE)

## Introduction

Invasive ant species are a major threat to the conservation of biodiversity on oceanic islands (Nashida & Evenhuis 2000; Wetterer 2002; Loope & Krushelnycky 2007; New 2008; Plentovich 2010; Rabitsch 2011). Yellow crazy ants (*Anoplolepis gracilipes*) in particular are one of the most invasive and ecologically damaging species (Holway et al. 2002). A. gracilipes is capable of invading natural environments and attaining extremely high densities that impose detrimental impacts to native insects (Gillespie & Reimer 1993; Hill et al. 2003; Lester & Tavite 2004), crustaceans (Green et al. 1999; McNatty 2009), reptiles (Haines et al. 1994), forest birds (Davis et al. 2008, 2010; Matsui et al. 2009), seabirds (Feare 1999; Plentovich et al. 2011), and trees (Hill et al. 2003; O'Dowd et al. 2003), which contribute to cascading effects on entire communities (Savage et al. 2009, 2011; Savage & Whitney 2011) and precipitate catastrophic changes to an entire rainforest ecosystem (O'Dowd et al. 2003). The documented loss of biodiversity and ecosystem integrity following A. gracilipes invasions coupled with the species' historic and global potential range expansion (Wetterer 2005; Chen 2008) make it a high-risk species (Stanley 2004) and justify its inclusion as one of the top 100 invasive species in the world (Lowe et al. 2004).

With an increasing number of natural areas being invaded by A. gracilipes (Wetterer 2005; Chen 2008) there is a pressing need to identify effective and efficient control tools that minimize non-target impacts. Historically, research on controlling pest ants has focused on a small number of species that have affected urban and agricultural settings (Williams 1993; Silverman & Brightwell 2008). While this work resulted in a range of products and techniques that effectively controlled target organisms, they are often unsuitable for use on invasions in conservation areas because they are ineffective on other ant species, have not been registered for such applications, or risks to non-target native organisms precludes their use (Krushelnycky et al. 2005; Rabitsch 2011). A. gracilipes supercolonies have been successfully controlled in Australia and Christmas Island in the Indian Ocean using Presto 01 Ant Bait (Bayer Environmental Science) containing the active ingredient fipronil (Green et al. 2004). However, Presto 01 Ant Bait is not registered for use in the United States (U.S.) and no other registered ant baits have been assessed for their efficacy against this species. This is not surprising as A. gracilipes has never been documented in the continental U.S., and has until recently only been found in the Hawaiian Archipelago and the Line Islands (Wetterer 2005) where its limited impacts (reviewed by Krushelnycky et al. 2005; Plentovich et al. 2011), have not engendered the focus of substantial management actions. However, in 2010 an A. gracilipes supercolony occupying approximately 50 ha was observed for the first time on U.S. soils at Johnston Atoll National Wildlife Refuge in the central Pacific Ocean (Flint & Woodward 2010). The absence of historically present Red-tailed Tropic bird (Phaethon rubricauda) nests within the invaded area, caused managers to suspect the ants as the cause of decline of nesting (U.S. Fish & Wildlife Service unpublished data).

With climate change models predicting an expansion of *A. gracilipes* by 2050 (Chen 2008), situations similar to the one at Johnston Atoll will become more common in the future.

The objective of this study was to test the efficacy of three commercial ant baits on the *A. gracilipes* population at Johnston Atoll. Based on results from a previous study evaluating the attractiveness of several baits to *A. gracilipes*, I selected Combat Source Kill Max A2 Ant Killing Gel (Combat), Maxforce Complete Brand Granular Insect Bait (Complete), and Maxforce Quantum Ant Bait (Quantum) to be examined in this experiment.

#### Methods

Study Site

Johnston Island is located within Johnston Atoll National Wildlife Refuge, an uninhabited, unincorporated United States territory. The atoll, located at 16.74°N, 169.52°W, has the distinction of being one of the most remote in the world. Though natural in origin, the island was artificially expanded to its current size of 253 ha and was the site of an active military base for nearly 65 years until its closure and abandonment in 2004. The current terrestrial environment is composed primarily of those plants and insects that have been able to survive since the abandonment of the previous intensively managed anthropogenic landscape (Amerson & Shelton 1976). The invaded area is dominated by *Pluchia indica* scrubland interspersed with a variety of ornamental trees and shrubs, cement building foundations, and asphalt roads.

# Plot Design

From within the approximately 50-ha infestation, 4 replicate 90×90 m experimental treatment plots (ETPs) were set up between 100 and 150 m apart. A block design was employed with each ETP consisting of four treatment subplots with monitoring transects running through them (Fig. 3.1). The 4 treatment subplots measured 30×30 m each and were arranged in a square with 30 m separating each from its two nearest neighbors. Treatment subplots were subdivided into 36, 5×5 m quadrats. A

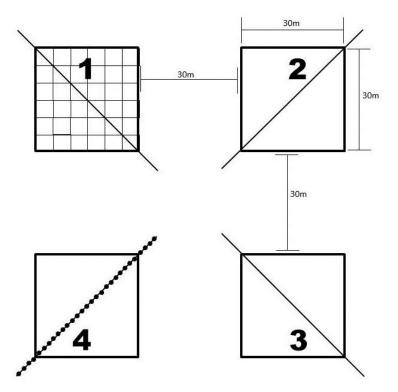


Figure 3.1: Experimental treatment plot (ETP) block design used to compare the efficacy of three commercial ant baits. Each of the four treatment subplots (large squares) were overlaid with a transect (diagonal black lines) of trap stations (black circles) running through the center and was subdivided into 5×5 meter quadrats (small squares in subplot 1) in which a prescribed amount of bait was applied.

monitoring survey transect extended through each subplot with monitoring stations placed every 2.5 meters resulting in 21 stations occurring inside each treatment subplot and 4 stations extending outside the treatment area on either end of the transect.

# Bait Application

A previous bait attractiveness study identified Maxforce Fine Granular Ant Bait (Fine Granular) as a being more attractive to A. gracilipes than Complete. However, during the course of the study it was learned that the manufacturer of the product had discontinued production of Fine Granular and it was no longer available. Complete was chosen as a substitute to replace Fine Granule in this study. Each subplot within an ETP was randomly assigned one of the three bait treatments and the remaining subplot was left untreated as a control. Complete and Quantum were manually broadcast while Combat was placed in bait stations. To ensure an even application rate of Complete and Quantum, each of the 36 quadrats within each treatment subplot were treated separately at the label prescribed application rates. Complete was manually broadcast at a rate of 0.15 oz (4.25 g) per 25 m<sup>2</sup>. Quantum, a syrupy liquid, was broadcast at a rate of 2.9 grams of bait per 25 m<sup>2</sup> and was applied by squeezing approximately 55, 0.05 g droplets directly on the substrate and vegetation. Combat applications involved 49 bait stations placed in a 5 m grid with approximately 2 ml of bait added to each station. Bait stations consisted of 15 ml centrifuge tubes left open and positioned on the ground and secured and shaded by either naturally occurring vegetation or the strategic placement of rocks or vegetative debris.

Each Complete and Quantum subplot had five bait availability monitoring stations haphazardly placed in areas judged to have high ant activity. Bait monitoring stations consisted of a 2 cm diameter plastic centrifuge cap placed under an inverted 10 cm diameter opaque plastic container that had 6 sections of the rim cut away to allow ants access. A single ~0.05 g drop of Quantum was placed on each cap in the Quantum

subplots and 10 individual granules of Complete were placed in the Complete subplots. Bait monitoring stations were checked each day and the estimated percentage of bait remaining was recorded. When the average amount of bait remaining in the 5 stations dropped below 50% the subplot was retreated following the initial procedure. Every time a subplot was retreated the bait monitoring station was reset. Combat bait stations were checked every day to ensure bait remained and bait was replaced as needed. If a station did not receive a bait refill within four days an additional 2 ml of fresh bait was automatically added to the station to ensure bait remained as attractive as possible. Complete and Quantum subplots were automatically retreated when rain occurred as soon as conditions allowed.

# *Monitoring of A. gracilipes*

Trap surveys: We conducted counts of A. gracilipes by using nontoxic baited traps consisting of 15 ml flip-top centrifuge tube with a pea-sized piece of SPAM placed inside. At first light each survey day, traps were placed at each monitoring station along the transects and left open for 2 hours, at which time they were closed, collected, and placed in a freezer for 48 hours after which the ants were identified and counted. Separate observers surveyed all four ETPs simultaneously. A pre-treatment trap survey was conducted on the same day but preceding the start of the treatment applications. Post-treatment trap surveys were conducted 12, 24, and 40 days after the beginning of bait treatment applications

<u>Presence absence surveys:</u> Baited monitoring station surveys are often biased toward dominant ant species and can fail to detect species that occur in low abundance (Bestelmeyer et al. 2000). If the pesticide treatments were to succeed in controlling A.

gracilipes it is possible that stations could fail to detect ants even if they are still present in the subplot. To address this issue an alternative metric will be measured that records the presence/absence of *A. gracilipes* in each of the 36 quadrats that make up each subplot. Each ETP was surveyed simultaneously for presence of ants shortly after first light each day. Observers performed an ocular examination of each 25 m² quadrat within each subplot for up to 1 minute. If no *A. gracilipes* were observed within one minute they were recorded as absent from the quadrat. The total time it took to complete each subplot survey was recorded. Presence/absence surveys were conducted within each subplot on the day of treatment, two days after treatment, and then every four days through the duration of the experiment.

# Data Analysis

The change in *A. gracilipes* abundance was examined by calculating the difference between the number of ants captured in the pre-treatment trap survey count and the number of ants captured in each of the three post-treatment trap survey counts at each monitoring station. The means of the differences within each subplot were calculated for each of the three post treatment survey days resulting in each bait treatment having a sample size of four for each of the three post treatment survey day. Treatments means were compared using a one-way analysis of variance (ANOVA) using Minitab 16 Statistical Software (2010). No analysis was performed on presence absence survey data due to the fact there was no change between pre- and post-treatment surveys with 100% of quadrats having *A. gracilipes* present in every survey.

#### Results

Bait application: The pre-treatment A. gracilipes trap survey was completed on the morning of 11 April 2011 followed by the initial treatment applications the same day. Weather conditions were fair for the first 6 days but frequent rains over the last 34 days required that all Complete and Quantum subplots be retreated 18 times due to rain washing away the bait. Rain did not impact Combat bait stations. Quantum and Complete bait monitoring stations were checked every day. The number of days in which the average amount of remaining bait in monitoring stations dropped below 50% and triggered a reapplication of bait occurred between 0 and 11 times with an average of 4.25 for Quantum subplots and between 2 and 9 times with an average of 5 times for Complete subplots. Out of the 196 Combat bait stations, only 3 required refilling outside of the automatic replenishing that happened every 4 days. A total of 544.65 oz of Complete was broadcast ranging from 124.2 to 162.0 oz in each subplot. A total of 12.8 kg of Quantum was broadcast ranging from 2.7 to 4.1 kg in each subplot. A total of 2.5

<u>Trap Surveys</u>: Out of a total of 2,063 traps, *A. gracilipes* were captured in 81%, no ants were captured in 18%, and other ant species were captured in just 0.004% of traps. Other species captured within the ETPs included *Tapinoma melanocephalum*, *Tetramorium bicarinatum, Monomorium floricola*, and *Cardiocondyla nuda*. All surveys were performed in dry conditions though rain did occur the previous day in the case of the 24- and 40- day surveys. Monitoring stations occurring outside the treatment areas were intended to make comparisons should ant numbers in treatment subplots be reduced

to undetectable levels. Since no treatment subplots were reduced to undetectable levels the stations that did not occur inside the treatment area were excluded from the analysis.

Twelve days after treatment, the average number of ants captured in each treatment group increased, with the exception of Quantum which decreased from 9.5 to 9.25 ants/station(N=84) (Fig. 5). The results of the one-way ANOVA revealed that the mean number of ants captured in the three treatments groups and the untreated group did not significantly differ from each other (F=0.233, df=3, p=0.8719). Subsequently, the average number of ants in each bait treatment subplot did decrease (Fig. 3.2), but none of the treatments significantly differed from the untreated control plot or from each other at either 24 days (F=2.793, df=3, p=0.086) or 40 days (F=0.199, df=3, p=0.895).

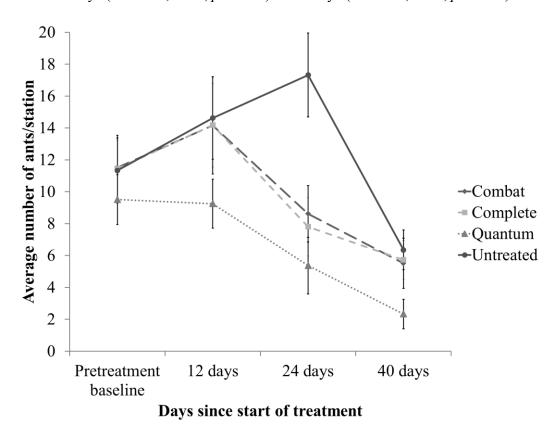


Figure 3.2. The average number with 95% confidence intervals of *A. gracilipes* captured per trap station for each treatment immediately prior to the initiation of treatment and 12, 24 and 40 days after the start of treatments.

<u>Presence/absence surveys</u>: There was no change in occurrence of *A. gracilipes* in any of the subplots on any of the 11 survey days. *A. gracilipes* was detected in 100% of the quadrats during every survey. Though no statistical tests are possible with constant measurement variables it is clear that none of the treatments reduced *A. gracilipes* populations to levels where they were no longer detected by bait stations or ocular surveys.

#### Discussion

Results of the efficacy test do not suggest that any of the selected baits are effective at controlling populations of *A. gracilipes*. Unfortunately, a long period of rainy weather that coincided with the last five weeks of the six week study does warrant some caution in interpreting the results. When rain was persistent enough to homogenously dampen the study area it would have also saturated the majority of Complete granules rendering them unattractive to ants, diluted or washed away an undetermined amount of the liquid Quantum bait, and substantially reduced foraging activity until standing water evaporated enough to no longer act as an obstacle to foraging ants. The effects of rain showers occurring on 18 of the last 34 days very likely overwhelmed and obscured the test's ability to detect any fine scale differences in the effects the bait treatments may have had in comparison to each other. However, as each of the baits tested are advertised to have observable impacts within a few days after just one application, and we repeatedly reapplied each bait over a 40-day period to ensure bait was available for at least a few hours of every day, I do feel confident that an observable affect would have

been detected had any of the baits reduced ant numbers to the level that would make them effective *A. gracilipes* control products.

The most compelling results supporting the lack of effectiveness of the tested baits are the increases observed in the average number of ants captured 12 days after treatment. Combat plots increased from 11.5 to 14.1 ants/station, Complete plots increased from 11.5 to 14.2 ants/station, and untreated control plots increased from 11.3 to 14.6 ants/station. The Quantum plots essentially stayed the same slightly decreasing from 9.5 to 9.3 ants/station. While rain occurred on 3 of the 12 days, the first 6 days remained dry and no obstacles were observed that would have prevented ants from foraging and all bait treatments were constantly available based on the observations made of the bait monitoring stations. In addition, during the initial six days no Combat bait stations went empty, Quantum subplots received between one and three reapplications and Complete subplots received between two and six reapplications. With only very low numbers of other competing ant species present in the treatment plots A. gracilipes is believed to have been the primary consumer of the baits. If any of the bait treatments had been effective the initial 6 day period alone should have sufficed to reduce A. gracilipes numbers.

The survey that occurred 24 days after the initiation of treatment is the only one of the three surveys that resembled results that one would predict when applying a pesticide to an ant population. The average number of *A. gracilipes* captured dropped for all three bait treatments by 39% (Combat), 45% (Complete), and 42% (Quantum) but their means did not significantly differ from each other nor did they differ from the untreated subplots which increased on average by 18% though the ANOVA test results

were only marginally insignificant (p=0.086). On the 40<sup>th</sup> day after treatment, all 3 treatment subplots decreased in the average number of ants captured by roughly the same amount, between 2 to 3 ants per station. However the untreated plot decreased in the average number of ants captured by 63% bringing the average number of ants down to be roughly equal to that of the treated plots (Fig 4). The crash in the untreated plots can likely be explained by very heavy rains that occurred four and three days prior to the survey which can reduce ant activity (Wirth & Leal 2001). This explanation would also have to be applied to the treatment plots since they would have been affected by the rain in the same manner causing reduced ant foraging activity and resulting in much lower counts of ants. While it is likely that the untreated plots would have had higher counts without the rain it is also likely that the treated plots would have had higher ant captures and any difference between untreated and treated may have still have failed to be significant.

Even though this experiment far surpassed the amount of effort that can be reasonably expected to be invested in a large scale management action, the most effective bait (Quantum) achieved no more than a 75% decline after 40 days of intensive efforts that included bait being reapplied between 18 and 30 days for each of the subplots. Regardless of the impacts of rain, from a manager's prospect, the experiment resulted in Combat, Complete, and Quantum failing to adequately reduce the numbers of *A. gracilipes* enough to warrant being recommended for further use.

#### **CHAPTER 4**

## IMPACTS OF ANOPLOLEPIS GRACILIPES

(HYMENOPTERA: FORMICIDAE) ON

RED-TAILED TROPICBIRDS (PHAETHON RUBRICAUDA).

## Introduction

Invasive ants are notorious for their direct and indirect effects on other organisms (Williams 1994; Moller 1996; Holway et al. 2002) including larger vertebrate species such as ground nesting birds (Drees 1994; Allen et al. 2000; Suarez et al. 2005). Common characteristics in the breeding biology of ground-nesting seabirds, including strong nest site fidelity and extended chick rearing periods (Bried & Jouventin 2002), make them particularly susceptible to negative impacts from invasive ants. Nest-bound chicks and brooding adults are susceptible to physical harm from invading ants for the simple reason that they need to remain at the nest site. Young chicks are physically incapable of moving, and abandonment by adults will result in the failure of the nest. Most seabird species also have part-time pair bonds, meaning the pairs do not spend the non-breeding season together and their previous nest site acts as a meeting point in order to reunite each breeding season (Bried & Jouventin 2002). Harassment from swarming ants can induce constant preening resulting in increased energy expenditure, which could lead to reduced nesting success (Vinson 1994) or simply the abandonment of the nesting site before mates can reunite. The low rates of population growth that characterize seabird populations leave them especially vulnerable to even small declines in breeding success that can have long-term consequences (Hamer et al. 2002).

Despite documented (Wetterer 2005) and predicted (Chen 2008) expansions of invasive ant populations, the impacts of invasive ants on seabirds are not well known. Currently, there are only 7 published accounts that describe interactions between invasive ants and seabirds (Lockley 1995; Feare 1999; Krushelnycky et al. 2001; McClelland & Jones 2008; Plentovich et al. 2009, 2011; DeFisher & Bonter 2013) half of which can be classified as anecdotal in nature. The paucity of data describing interactions between invasive ants and seabirds is in part due to the difficulty of studying species that have low fecundity (Weimerskirch 2002) as well as the asynchronous and aseasonal breeding that is common in seabirds and results in highly variable demographic parameters from one year to the next (Citta et al. 2007). This high variability can potentially mask all but the most severe effects exhibited on seabird populations by invasive ants. When one takes into account the additional complication that most seabird colonies occur in areas inaccessible to mammalian predators and human observers, (Burger & Gochfeld 1994; Coulson 2002) it is no mystery why so little data has been generated.

The discovery of a yellow crazy ant (*Anoplolepis gracilipes*) supercolony at Johnston Atoll National Wildlife Refuge is a case in point. Johnston Atoll is one of the most remote areas in the world containing the only terrestrial habitat in over 850 thousand square miles of open-ocean, making it a critical breeding habitat for 15 seabird species. Due to its remoteness, biologists with the Unites States Fish & Wildlife Service (Service) have been restricted to monitoring expeditions of just three to six days once every two years. On one of these visits in January 2010, Service biologists arrived to discover an *A. gracilipes* supercolony covering an estimated 50 ha area of Johnston Island. The density of the supercolony was so high it was suspected that the ants were

reducing the suitability of nest sites and altering the behavioral patterns of ground-nesting Red-tailed Tropicbirds (*Phaethon rubricauda*).

Managers responded to the crisis by deploying a strike team to Johnston Atoll that began working towards controlling the *A. gracilipes* supercolony by using a variety of chemical ant baits. While there is little doubt about the negative impacts the ants were having on the *P. rubricauda* nesting population, the urgency of the situation did not allow for the limited resources available to be devoted to anything other than ant control measures. Documentation of the seabird impacts were limited to anecdotal assessments made by the strike team which found that throughout the following 18-months of control efforts when *A. gracilipes* abundance remained extremely high, no more than an estimated 8-12 *P. rubricauda* nests were located within the 50 ha infestation area and these were typically found along the perimeter where ant abundance was less consistent (SJK-personal observation, USFWS unpublished data).

By January of 2012 the strike team had succeeded in reducing the abundance of *A. gracilipes* by 99% (USFWS-unpublished data). The resulting decrease in ant control efforts allowed for resources to be devoted to a structured examination of the impacts the ants were having on *P. rubricauda* nesting. Immediately following the reduction in *A. gracilipes* numbers, I was able to explore the occurrence of *P. rubricauda* nesting on Johnston Island with the goal of obtaining quantifiable information that could be used to make comparisons between the number of nests found in areas in which *A. gracilipes* was never detected and areas in which the *A. gracilipes* supercolony had been recently controlled. Here I present the results of a comparison that tested the hypothesis that the occurrence of *A. gracilipes* reduces the number of *P. rubricauda* nests.

#### Methods

Study Site

Johnston Island is located within Johnston Atoll National Wildlife Refuge, an uninhabited, unincorporated United States territory. The atoll, located at 16.74°N, 169.52°W, has the distinction of being one of the most remote in the world. Though natural in origin, the island was artificially expanded to its current size of 253 ha and was the site of an active military base for nearly 65 years until its closure and abandonment in 2004. The current terrestrial environment is composed primarily of those plants and insects that have been able to survive since the abandonment of the previous intensively managed anthropogenic landscape (Amerson & Shelton 1976). The invaded area is dominated by *Pluchia indica* scrubland interspersed with a variety of ornamental trees and shrubs, cement building foundations and asphalt roads. The un-invaded areas of the island are similar, though in general there are far fewer ornamental trees and patches of *Casuarina equisetifolia* cover a larger proportion. *P. rubricauda* typically nest throughout the island in any available shade.

Study Plots

As part of the ant control and monitoring project, a 50m grid was laid out over the entire island consisting of 1,052 individual stations in which multiple environmental variables had been recorded including substrate composition, vegetative cover and vegetative height classes. From this set of stations I excluded those in areas composed of over 30% cement or asphalt ground cover, had less than 10% vegetative cover, and that occurred within 50 meters of the most recently measured infestation perimeter or the coast line. The remaining stations were divided between those occurring inside the

infested area (n=50) and those occurring outside (n=245). Thirty stations were randomly selected from within each set and those stations became the center points of 40×40 meter nest plots.

#### Data collection

In relation to the changing status of the supercolony, the nest count surveys began approximately 1 month after ant numbers had been reduced by 80% and within 1 week of ant number decreasing by 99% of the pre-treatment baseline. The timing meant all nests with chicks counted in the first survey would have been initiated prior to the 80% reduction in ant abundance when numbers were reduced but still substantial, and all nests with eggs would have been initiated during the period the ant numbers were dropping from around 80% to 99%.

Each plot was visited and searched for active nests containing an egg or chick.

All nests were confirmed to be active by tipping the adult if a chick was not observed from a distance. Nests with unattended eggs or adults tending a nest without an egg or chick were not counted. For each active nest the date, species, dominant vegetation type, and nest status (egg or chick stage) was recorded.

## Data Analysis

For any chicks to have been present in the survey, adult pairs would have had to have laid and incubated eggs prior to the 99% reduction in *A. gracilipes* numbers though the primary decline would have begun with implementation of a new bait formula in November 2011. The total number of chicks in each plot was calculated and Minitab 16 Statistical Software (2010) was used to perform a Mann-Whitney U test to compare the medians of the number of chicks in plots inside and outside the infested area.

#### Results

The surveys occurred between 22-January and 22-February 2012. A total of 72 P. rubricauda nests (59 with egg; 13 with chicks) were counted in the ant infested plots and a total of 136 nets (98 with eggs; 136 with chicks) were counted in the plots with no A. gracilipes present. The median (M) number of chicks found in the infested plots (M = 0) was significantly fewer than the number counted in the un-infested plots (M = 1.5)(Mann-Whitney U test: U = 245.5; z = -3.02; p = 0.001).

### **Discussion**

When *A. gracilipes* was identified for the first time on Johnston Island in January 2010, it had already displaced nearly all ground nesting seabirds within an estimated 50-ha area where there had previously been hundreds of Red-tailed Tropicbird (*Phaethon rubricauda*) nests. The direct mechanism by which the birds were driven away from their traditional nesting sites appeared to be harassment endured from the constant swarming of ants and, even more severely, the blinding of the birds caused by the formic acid the ants spray. *P. rubricauda* that were observed to have remained in the infested area over night, presumably attempting to reunite with their mate, were observed to be stressed and agitated with disheveled feathers stained brown from formic acid and nictitating membranes that were completely swollen shut leaving the birds blind. The few observations made of birds attempting to take flight typically resulted in aborted take offs and violent impacts with nearby vegetation which confirmed that birds were unable to see (SJK-personal observation). Birds located outside of the infested area exhibited no signs of disturbance and occurred in the anticipated historic densities (USFWS)

unpublished data). The ultimate result of the ant's impacts on the *P. rubricauda* population appeared to be the complete abandonment of the nesting grounds within the *A. gracilipes* infested area

The results of this study support the hypothesis that the presence of A. gracilipes reduces nesting of *P. rubricauda*. Overall, fewer nests initiations occurred in plots with ants present than in plots that never had ants. Plots in which A. gracilipes had never been detected had a total of 98 P. rubricauda nests with chicks in them (mean = 3.27) chicks/plot) compared to a total of 13 chicks found in an equal number of ant infested plots (mean = 0.43 chicks/plot). Despite the survey being conducted after the 99% decline in ant numbers, this survey demonstrates the impact of the presence of A. gracilipes because any chicks present in the survey period had to have been incubated by their parents prior to the 99% reduction in ant numbers. Of the 13 chicks counted within the infested plots during this survey, 12 were estimated to be less than one week old. Given the mean incubation period of 43-days (Dearborn & Anders 1996), the earliest date the eggs that produced these chicks were likely to have been laid was 3-December-2011. The initial bait treatment had been ongoing for about one month prior to that date and surveys indicated that A. gracilipes numbers had declined by 30% on 30-November and had declined by 60% by 14-December. It is likely that these 12 nests that were initiated prior to the 99% reduction in A. gracilipes numbers had done so in response to the decreasing abundance of ants that occurred in response to the bait treatments. In comparison, the 98 chicks counted in the non-ant infested plots were not just significantly greater in number, but included chicks that spanned all age classes including 13 that were estimated to be over 8-9 weeks old and were near fledging. Though *P. rubricauda* show

a regular peak in nest initiations each year around March (Schreiber & Schreiber 2009) they are found nesting throughout the year on Johnston and the variation in chick ages found in the non-ant infested plots is typical of what one would expect to find at any given point in the year (USFWS-unpublished data). While this comparison did result in a significant difference between infested and non-infested plots, if the surveys could have been completed in the months prior to the bait treatments when ant numbers remained exceptionally high it is likely the resulting differences would have been even greater, supporting the anecdotal observations of an almost complete lack of *P. rubricauda* nests within the infested area.

The results of this study provide quantitative support for the anecdotal observations that populations of *A. gracilipes* can result in the physical displacement of ground-nesting seabirds like *P. rubricauda*. While the apparent growth in the *P. rubricauda* nesting colony at Johnston Atoll following ant control efforts is encouraging (USFWS-unpublished data), the continued presence of *A. gracilipes* on the island remains a concern. Until the *A. gracilipes* population is eradicated it will continue to be an ever present threat and without continued control efforts will most likely regain the high ant densities that displaced ground-nesting seabirds.

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