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Management and Monitoring of Linepithema humile (Mayr) on San Clemente Island, CA

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Management and Monitoring of Linepithema humile (Mayr) on San Clemente Island, CA

A Thesis submitted in partial satisfaction of the
requirements for the degree of

Master of Science

in

Entomology

by

Korie C. Merrill

June 2015

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DEDICATION

To the ants that gave their lives for research.

ABSTRACT OF THE THESIS

Management and Monitoring of Linepithema humile (Mayr) on
San Clemente Island, CA

by

Korie C. Merrill

Master of Science, Graduate Program in Entomology
University of California, Riverside, June 2015
Dr. Erin Wilson Rankin, Co- Chairperson
Dr. Dong-Hwan Choe, Co-Chairperson

The Argentine ant, *Linepithema humile* (Mayr), is an extremely invasive ant species that has spread to urban, commercial and natural areas worldwide. This pervasive expansion has had detrimental ecological and economic effects, resulting in the allocation of vast amounts of resources to its control in urban and agricultural areas. New efforts are underway to control Argentine ants in ecologically sensitive habitats, such as California's Channel Islands. I tested the treatment efficacy of application of thiamethoxam liquid bait in ecologically sensitive habitats using polyacrylamide beads as a delivery matrix to eradicate Argentine ants. This matrix is shown to be a promising eradication tool on San Clemente Island, CA with a 99.86% reduction in Argentine ant activity across five sites (176.95 hectares). As these eradication efforts are implemented, a standardized detection protocol becomes essential to gauge the success of such efforts and to ensure that remnant ant populations don't go undetected during pre- or post-treatment stages. In aid of creating such protocols, I conducted field trials to assess 1) attractant efficacy for Argentine ant detection throughout the year and 2) pre- and post-treatment detection rates

of Argentine ants. Traditional sucrose water bait traps can be enhanced with the addition of sugar-egg or a synthetic pheromone of the Argentine ant. The sugar-egg and pheromone bait traps performed equally well at detecting Argentine ants in areas with low levels of ant density. This information will be used to standardize detection protocols in a diversity of ecosystems and to refine Argentine ant eradication efforts on the Channel Islands.

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CHAPTER I. Introduction

San Clemente Island (SCI) is the most southern of California's Channel Islands Archipelago, situated approximately 125 km west of San Diego and 100km south of Long Beach, California (Fig. 1). Since 1934, SCI has been owned and managed by the US Navy. San Clemente Island lays claim to 14 endemic plant species (Beachamp, 1987; Raven, 1963), six of which are federally listed; two endemic terrestrial mammals; four species of bats (Brown, 1980); two endemic bird species (Jorgensen and Ferguson, 1984) and 13 native ant species (David Holway, *pers. comm*.). In addition, SCI hosts the invasive Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera; Formicidae) in the northern half of the island.

Native to South America, Argentine ants have been introduced worldwide and now persist on many islands and all continents except the Antarctic, resulting in numerous detrimental ecological impacts (Harris, 2002; Suarez *et al.*, 2001, 2000). This invasive species alters biological communities by negatively affecting native ant populations (Holway, 1998; Human *et al.*, 1998), other invertebrates (Lach, 2007), plants and pollinators (Hanna *et al.*, 2014; LeVan *et al.*, 2014), and even terrestrial vertebrates (Suarez *et al.*, 2001). In conjunction with Argentine ant's high abundance and aggressive response towards other arthropods, especially other ants, their ability to quickly dominate resources might have given them a competitive advantage over native species (Human *et al.* 1998; Holway and Case 2000; Harris *et al.* 2002). This is particularly a problem in California's Channel Islands where Mediterranean ecotypes host a diverse group of species (Cowling *et al.*, 1996; Jenkins *et al.*, 2015).

Although Argentine ants were first recorded in California in 1907 (Woodsworth, 1908), they were not documented on San Clemente Island until 2008. The maritime climate of California's Channel Islands might have favored the spread of this exotic species within these locations (Holway *et al.*, 2002; Suarez *et al.*, 1999). While successful in disturbed areas, they can effectively invade surrounding natural habitats as well; examples include coastal sage scrub and riparian woodlands in mainland California (Holway, 1998; Suarez *et al.*, 2001). On SCI, most of the known infestations are spatially and temporally associated with development activities on the island; it is likely that Argentine ants were first transported to the island via various construction materials.

In 2009, The Nature Conservancy (TNC) in collaboration with the National Park Service (NPS) started an Argentine ant eradication project on Santa Cruz Island (Boser *et al.* 2014). This project used the novel technique of aerial application of sugar water plus thiamethoxam imbibed in hygroscopic crystals (Boser *et al.*, 2014; Rust *et al.*, 2015). Following the success of the Santa Cruz Island pilot project the U.S. Navy determined that it was feasible and necessary to implement an Argentine ant eradication project on San Clemente Island to protect its natural resources. Eradication, the complete removal of a species from a particular area, should be the considered before control, the maintenance of a small population size. Although both can be logistically and economically costly, eradication is preferred since it is implemented once instead of in perpetuity; thus if effective it is overall more cost effective than control (Courchamp *et al.*, 2003).

A critical part of an effective eradication program is accurate mapping of an infestation or infestations. This is of particular concern for this project as Argentine ants are difficult to survey and detect due to their small size and fluctuating population densities across seasons and years (Ward and Stanley 2012). Inaccurately mapped or incomplete mapping of infestations can increase costs and otherwise undermine the success of eradication efforts. Even with the ongoing eradication program on SCI, the invasion of Argentine ants have been expanding in some areas of the island. For example, in 2013 organized searches of structures and habitat restoration sites were conducted to delineate the Argentine ant infestation on SCI resulting in seven infested sites totaling 357.14 ha (884 ac). Since 2013, an additional 56.97 ha (141 ac), were found to be infested with Argentine ants, including three new sites plus augmentation of previously known sites. The Wilson Cove infestation on San Clemente Island expanded in three years (2011 - 2013) at a rate of 30m per year. This range expansion increased the total acreage of the Wilson Cove infestation by 55.75 ha (138 ac) (Fig. 2). At an estimated eradication cost of $1900/ha, this expansion of Argentine ants is not only ecologically costly but economically as well.

As with many eradication efforts, management efforts (removal) need to be followed by extensive monitoring to find remnant individuals or populations. Accurate detection of the few remaining nests is often challenging, since resurgence of small surviving populations can result in failure of the eradication program (Hoffmann, 2011). It is these few, isolated remnant nests that are the most difficult to eradicate because small numbers of individuals are difficult to find (Courchamp *et al.*, 2003). It is often difficult to prove that a species was not recorded because it truly was absent instead of simply not being detected (Gu and Swihart, 2004; Wenger and Freeman, 2008). The second part of this study (Chapter 3) focuses on non-toxic bait preference for pre- and post-treatment monitoring for large scale Argentine ant eradication projects.

The information obtained from these studies will be used to standardize Argentine ant detection protocols and future prevention plans in a diversity of ecosystems. The first part of this study (Chapter 2) outlines the implementation of an Argentine ant eradication project on San Clemente Island using aerial deployment techniques developed by TNC on Santa Cruz Island, CA. It was expected that a combination of aerial, hand and all-terrain vehicle (ATV) application of thiamethoxam bait would be effective at killing Argentine ants on San Clemente Island. A traditional monitoring technique was used to determine the abundance of Argentine ants in infested areas on SCI. From this data, detection probability of Argentine ants was calculated using techniques described in Wegner and Freeman (2008). The results of this study show that aerial application of liquid thiamethoxam bait is effective in controlling field populations of Argentine ants in large natural areas.

The second study (Chapter 3), aims to improve the traditional monitoring techniques for detecting Argentine ants, thus increasing the probability of detection. In particular, monitoring at low densities, either in areas with relatively new infestations or an ongoing control program can be challenging (Hoffman 2011). The study indicated the addition of protein or Argentine ant pheromone increased baits attractiveness over the traditional 25% (w/v) sucrose solution. The new bait trap techniques will be valuable to increase detection confidence of Argentine ants for management/eradication programs.

Figures Chapter I.

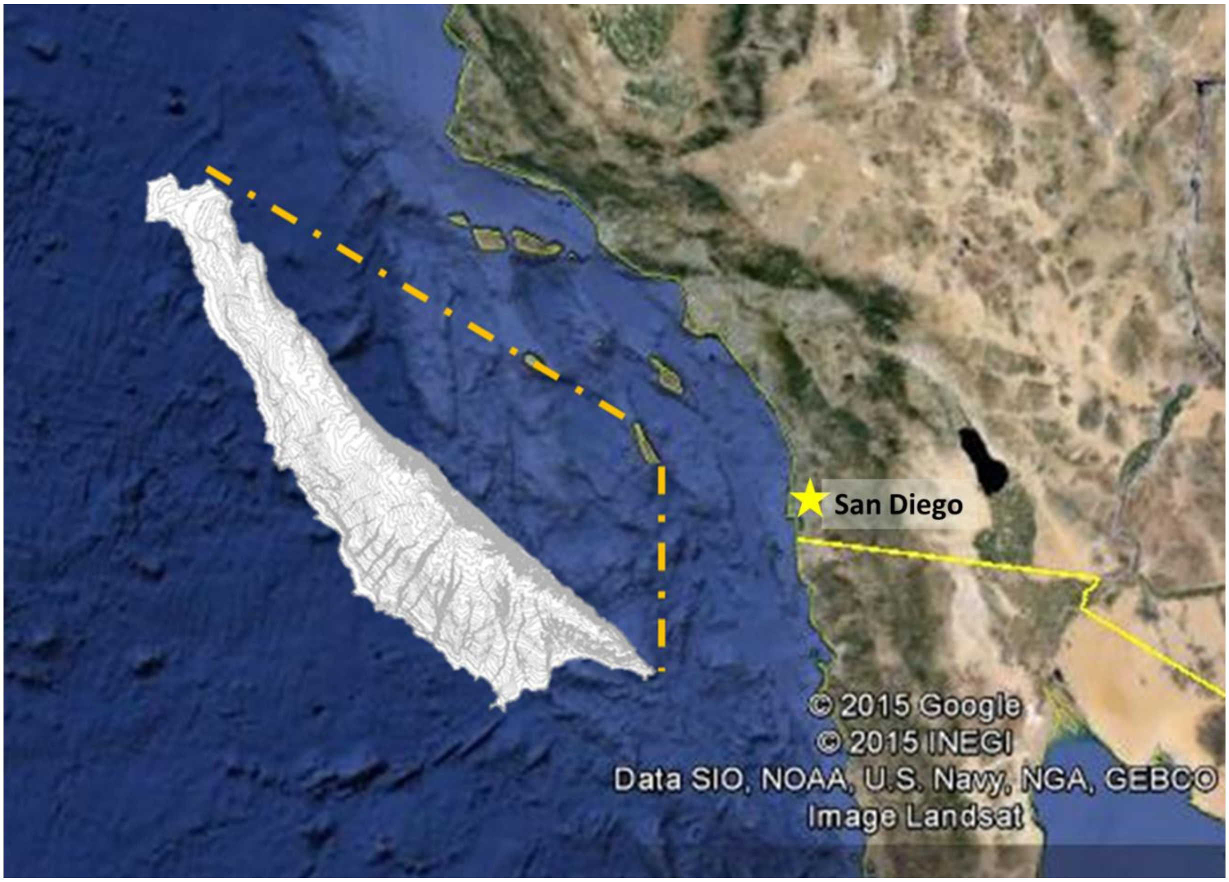

Figure 1. Map of California Coast. Magnified view of San Clemente Island, located approximately 125km off the southern California coast. The island is 35km long and 6.67km at its widest point totaling an area of $56km^2$. The yellow star represents San Diego, CA.

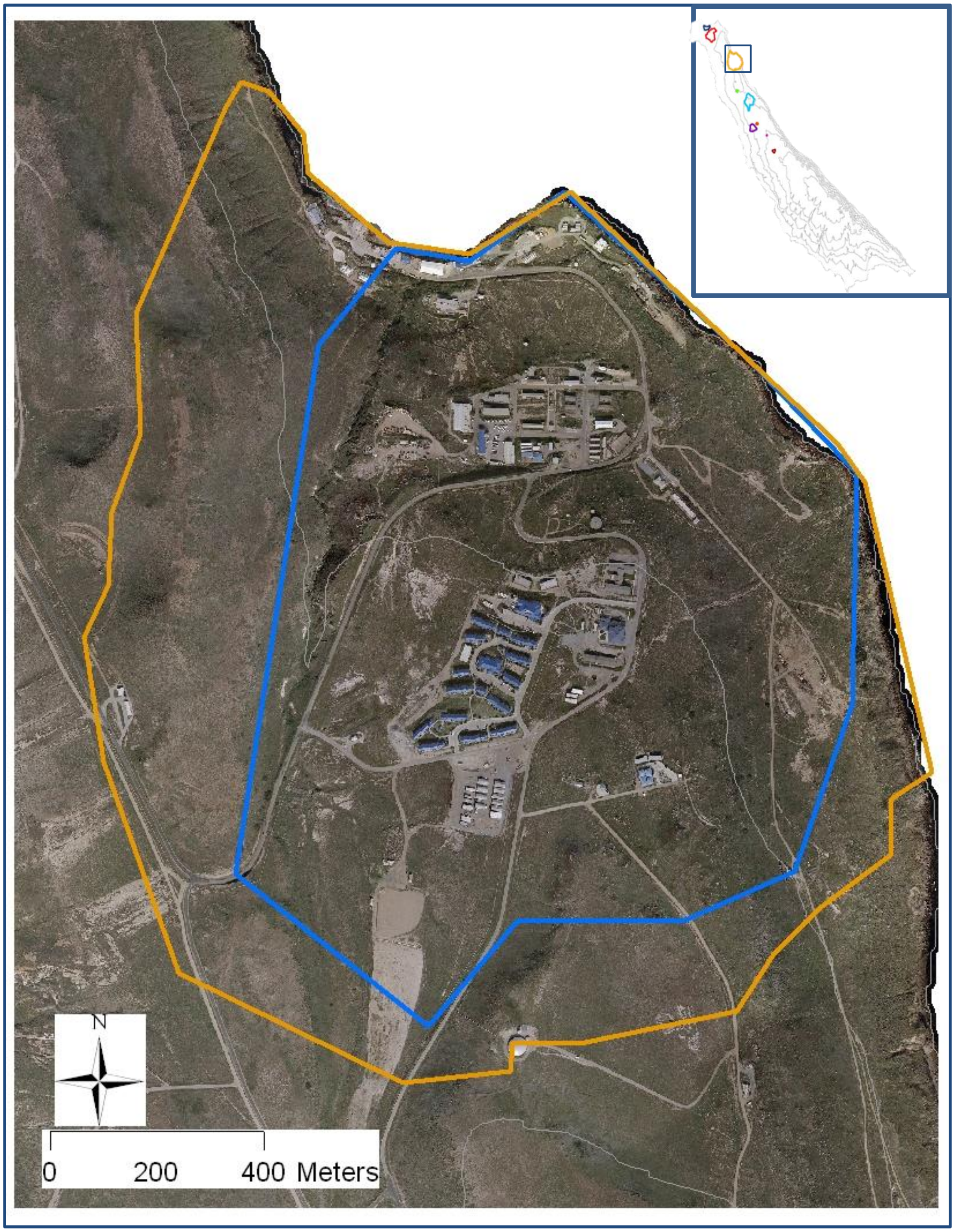

Figure 2. Map of infested site. Argentine ant expansion at Wilson Cove from 2011 (blue polygon) to 2013 (yellow polygon). This expansion increased the infested area by 55.75 ha.

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CHAPTER II. Abundance and detection probability of invasive Argentine ant on San Clement Island after eradication efforts

Introduction

An estimated 10% of all introduced species will establish in new areas, of which 10% of those will be ecologically destructive (Williamson and Fitter, 1996). The Argentine ant (*Linepithema humile*, Mayr) is one of those ecologically destructive species (Holway, 1998; Sanders *et al.*, 2001; Suarez *et al.*, 2000). This invasive species disrupts ecologically sensitive areas by negatively affecting native flora and fauna (Hanna *et al.*, 2014; Holway, 1998; Human *et al.*, 1998; Lach, 2007; LeVan *et al.*, 2014; Suarez *et al.*, 2000). This is particularly a problem on California's Channel Islands which host a diverse group of endemic and rare species (Jenkins *et al.*, 2015). San Clemente Island (SCI), the most southern of California's Channel Islands archipelago that hosts a number of endemic plant and animal species, seven of which are listed as federally endangered; (Raven 1963; Jorgensen & Ferguson, 1984; Beachamp 1987). In addition, SCI has been invaded by the Argentine ant in the northern half of the island (Fig. 1). Although the infestations only overlap with one rare plant species (*Acmison dendroideus traskiae*) at one location, and one rare bird species (San Clemente Island Bell's Sparrow), the potential threat if ants spread further into endangered species' habitat is formidable. Of particular concern was the site Wilson Cove, where Argentine ants infested the island's native greenhouse and nursery. Container plants from this nursery and greenhouse are regularly transplanted at habitat restoration sites throughout the island; thus increasing the probability of introducing Argentine ants into new areas. Because of the potential spread of this destructive species, the U.S. Navy decided to take actions to eradicate Argentine ants from San Clemente Island in 2013.

Historically, eradication of invasive species is difficult and often costly with only a small percentage resulting in successes and most only successful on islands (Courchamp *et al.*, 2003). In most success stories, the target species are mammals, very few are plants and even fewer documented invertebrate species (Courchamp et al 2003; Howald et al 2007, Hoffman 2010). Ants are particularly problematic, with few published successful eradications as of 2010 ((Hoffmann, 2010) 2010). Reasons for failed eradications include: (i) limited funding and support to see the project to completion, (ii) missed or untreated populations, (iii) reintroduction from an outside source, (iv) a lack of knowledge of the biological system of the target species, and (v) inadequate bait delivery systems (Courchamp *et al.*, 2003; Hoffmann, 2010; Krushelnycky and Reimer, 1998; Rust *et al.*, 2000).

Argentine ants are particularly difficult to eradicate because they are polydomous (more than one queen in a nest) and unicolonial (cooperation of multiple nests in a single colony) and form high density infestations that can quickly populate new areas (Holway 2002; Tsutsui and Suarez 2003; Silverman and Brightwell 2008). A novel baiting technique on Santa Cruz Island, CA has revolutionized large-scale eradication of this invasive ant species using hygroscopic polyacrylamide beads deployed via a helicopter (Boser *et al.*, 2014).

Even with this new treatment technique, most of the efforts, time and thus money, will be spent on finding and controlling the few remaining individuals or nests after treatment (Courchamp *et al.*, 2003; Hoffmann and O'Connor, 2004; Howald *et al.*, 2007). For this reason, it is essential to have both a plan to transition from treatment to monitoring and effective monitoring and detection protocols in place (Courchamp *et al.*, 2003). To increase the confidence of monitoring or detection protocols, determining detection rates of Argentine ants is critical (Ward and Stanley 2013). Even with the known importance of monitoring in eradication and control projects, there is little documented research on standard monitoring and detection protocols for ants (Ward and Stanley 2013). In this study, occupancy modeling was used to improve and standardize monitoring protocols across the Channel Islands.

Treatment protocols implemented on San Clemente Island, described here, were assembled from previous work conducted by The Nature Conservancy (Boser *et al.*, 2014; Rust *et al.*, 2015). Presence-absence and ant activity data from monitoring efforts on San Clemente Island across five sites (610 points in total) during the 2014 season was used for modeling Argentine ant detection rate, occupancy and abundance.

Methods and Materials

Eradication Program.

Based on efficacy experiments conducted by The Nature Conservancy and Dr. Mike Rust at the University of California Riverside, toxicant bait consisting of Optigard® Flex Insecticide (Syngenta Crop Protection LLC; 21.6% thiamethoxam) diluted with 25% (w/v) sucrose water solution to 0.0006% (6PPM) thiamethoxam was used to control populations of Argentine ants (Rust *et al.*, 2015). Hygroscopic polyacrylamide beads (Magic Beads Inc., Miami, FL) were added to this formulated thiamethoxam and 25% sucrose solution until the beads were fully hydrated and reached an individual mass of 1.03 ± 0.15 g typically 24 hours after mixing (Fig. 2). The physical structure of the hygroscopic polyacrylamide bead holds liquid bait in place, allowing it to be broadcast over a large area (Boser *et al.*, 2014; Buczkowski *et al.*, 2014). As per the Optigard® Flex Insecticide label restrictions described in the Experimental Use Permit (EUP), bait was mixed as needed to broadcast at a rate of approximately one polyacrylamide bait bead per square foot and approximately 0.013 L/m^2 (14 gallons per acre) but not to exceed the rate of 0.015 L/m^2 (16 gallons per acre). Bait was mixed at least 24 hours prior to deployment to allow for full absorption of the thiamethoxam solution and staged overnight onsite.

In 2014, five sites on SCI (Fig. 1) (totaling 176.95 hectares) infested with Argentine ants were treated with the thiamethoxam beads 7-9 times at an application rate of 0.013 L/m^2 as per label instructions. Three of the sites (Wall, VC3 and BUDS) were treated using a combination of hand and ATV deployment protocols. Wall and VC3 were treated earlier (April-November) than BUDS (August-November) due to restricted access to the latter site.

To treat the coastline at BUDS, bait stations were deployed twice during the summer instead of beads. Bait stations were placed 50m apart in a singular transect along the beach and left for one week. These bait stations consisted of 15 ml centrifuge tubes (Celltreat Polypropylene Centrifuge Tube, Celltreat Scientific Products, Shirley, MA) filled with 10ml of thiamethoxam solution and two to three beads to retain moisture in the tube for multiple days.

The perimeters of roads and buildings (30m buffer) at the Airfield site were also treated by a combination of hand and ATV deployment. To deploy bait by hand, technicians walked in parallel lines, approximately 6-9m apart to avoid an overlap of more than 3m in bait distribution, while distributing bait using a chest handheld broadcast spreader (EarthWay ®) with a capacity of 18kg (Fig. 3a). To treat large areas along roads and inside the Airfield taxiway, we used an ATV (Honda) with an 11-gallon motorized hopper with a deployment swath of 12m (Fig. 3b). Two sites, Dump and Airfield, were treated (August-November 2014) aerially by Aspen Helicopters, Inc. using a 284 L (75 gal) hopper long-lined from a helicopter. Both sites were treated in one day with the total duration varying from 8h to 6h of flying time per deployment. The pilot, via adjustment of the hopper aperture, controlled the rate of bait application. Each load of bait was distributed across the site with a swath of 40m. To ensure complete coverage of infested sites, tracks of areas treated were recorded with handheld GPS units (Garmin eTrex 20, Garmin Ltd. Olathe, KS) (Fig. 4), or an airplane GPS unit controlled by the pilot and mapped using ArcMap 10.2.2 (Esri) (Fig. 5).

Abundance and Treatment Efficacy.

To test the efficacy of the bait application, point count monitoring was conducted before and after treatment at all treated sites (Airfield, Dump, Wall, VC3 and BUDS) plus two control sites (Wilson Cove and Magazine) (Fig. 1). Control sites, or areas with Argentine ants present but without pesticide treatments, were chosen based on the presence of Argentine ants, site accessibility, and size such that there were approximately the same number of total points (~400) at the control sites as treated sites. Monitoring points were overlaid in a grid on the infestation area of each site treated in 2014 (Fig. 6). At small sites (<12ha) such as VC3 and Wall, points were spaced 20m apart whereas monitoring points were spaced 50m apart at the larger BUDS, Dump, Airfield, Wilson Cove and Magazine (>12ha). The difference in spacing at larger and smaller sites was done to ensure these smaller sites had adequate points to detect differences in Argentine ant activity. All sites were monitored twice before pesticide application, once during the treatment season and once at the end of the season—two weeks after the last deployment (Table 1.). Technicians navigated to each point using handheld GPS units. In the best-suited habitat, within 5 m of each established point, technicians placed monitoring traps, a 50mL centrifuge vial (Celltreat) containing a cotton ball soaked with 25% (w/v) sucrose water (~4.5g). Ideal habitat included any shrub, sub-shrub or thick ground cover *i.e.* *Opuntia littoralis* (Prickly-pear Cactus), *Calystegia macrostegia* var. *amplissima* (Island Morning Glory), beneath thick grass (typically *Bromus* species) and *Atriplex semibaccata* (Australian Saltbush), *Baccharis pilularis* ssp. *consanguinea* (Coyote Bush) and *Rhus intergrifolia* (Lemonade Berry). Each vial was labeled with the point number at which it was placed. After 1-1.5 h, vials were collected, frozen and then the numbers of ants present per point were counted.

Statistical Analysis.

All data were analyzed using R v.3.1.2 (The R Foundation for Statistical Computing, 2014) unless otherwise specified. Total ant activity before and after treatment was analyzed using a paired t-test in the "stats" package (R Core Team and contributors worldwide, 2014). Presence-absence data at treated sites was analyzed using Pearson's Chi-Square test in the "stats" package.

To estimate Argentine ant abundance, occurrence and detection probability, a zero-inflated Poisson model was used, which accounts for three factors: abundance, occupancy and incomplete detection (Wenger & Freeman 2008). This was done by using repeated per-point estimates based on point-level resampling aggregated to site (Wenger and Freeman, 2008). Data from pre- and post-treatment monitoring rounds from all sites were combined for analysis with pesticide treatment as a covariate for occurrence and abundance.

Results

Argentine ant activity was significantly reduced at most sites, except Wall (Wall: 3.03 vs 0 ants/point, t=1.52, df =41, p=0.14). The average number of Argentine ants per point across all five treated sites was reduced by 99.86% (62.57 to 0.09 ants/point) after seven treatments (t=4.54, df =385, p<0.01). At the BUDS site, no Argentine ants were detected before or after treatment at the established 29 monitoring points (Table 2). The Airfield had the most reduced activity (208.16 vs 0.19 ants/ point, t=4.70, df =168, p<0.01), followed by VC3 (3.55 vs 0 ants/point, t=2.22, df =32, p=0.03) and Dump (25.90 vs 0 ants/point, t=3.34, df =85, p<0.01). At these treated sites, Argentine ants were detected at 23.99% (130/484) of points before treatment and <0.01% (2/415) of points after treatment (X^2 = 94.80, df =1, p<0.01). The two points with Argentine ant present during post-treatment monitoring in November 2014 were both in the Airfield site (Fig. 7). At the control sites (Wilson Cove and Magazine), Argentine ants were detected at

8.21 % (16/195) of points during the first monitoring round in July and 9.64 % (19/197) during the last monitoring round in November (X^2 = 0.25, df =1, p =0.67).

The number of pesticide deployments negatively affected the probability of Argentine ants to occur in an area (Fig. 8), thus the zero-inflated Poisson model is consistent with the results from the previous treatment efficacy results. Probability of occurrence precipitously drops after the first pesticide deployment however occurrence doesn't drop below 0.1 until 8 deployments.

Conclusions

Within ten minutes after thiamethoxam-laced bait beads were deployed, Argentine ants were seen foraging on the bait beads (Fig. 9); however after 24 hours no Argentine ants were seen on bait beads most likely due to evaporation of water which makes the beads viscid the following day. This is consistent with previous lab studies showing that hygroscopic baits lose attractiveness to ants after 9h at 0-33% relative humidity (Rust *et al.* 2015).

A small proportion of monitoring points were only visited once due to area restrictions for training activities. BUDS was particularly problematic with only 29 monitoring points visited twice. No Argentine ants were recorded at BUDS (before or after pesticide treatments); this is likely due to the location of the monitoring grid within the site (along the coast line) and the small number of sampling points (n=29). At this site, the use toxicant bait stations was a preliminary study of whether or not this type of toxicant bait station could be used for treating ecologically sensitive areas such as water edges as well as in high use areas to minimize non-target effects. Targeted monitoring at this site especially of areas closer to buildings and shrubs is warranted. At other sites, even if monitoring points were accessible for multiple monitoring rounds, baits were removed by the inquisitive San Clemente Island Fox (*Urocyon littoralis* ssp. *clementae*). If areas are susceptible to interference from animals, monitoring traps should be fixed to a point or a larger monitoring trap design should be used to reduce movement.

The only monitoring points with residual ant activity after seven deployments were at the Airfield site. Both of the points had Coyote Bush nearby; no Argentine ants were found in open areas (i.e. annual grass or bare ground) after pesticide treatment. This suggests that targeted pesticide application to shrubs, Coyote Bush in particular, during treatment may be warranted on SCI.

Four of the sites on San Clemente Island were treated seven times and only one site (VC3) was treated nine times. On Santa Cruz Island, sites were treated 12 times in 2013 and twice in 2014; after one season of monitoring, no Argentine ants have been found in these treated areas (Boser *et al.*, 2014). In areas with higher ant activity, more than seven treatments are necessary to fully eradicate this invasive species in one season using the protocols described here.

Further research on developing monitoring protocols is also needed to improve the detection of Argentine ants especially in low-density areas, such as after eight deployments when probability of occurrence is at 1% when the last remaining individuals are the most difficult to find (Courchamp *et al.*, 2003; Hoffmann, 2010).

Figures Chapter II.

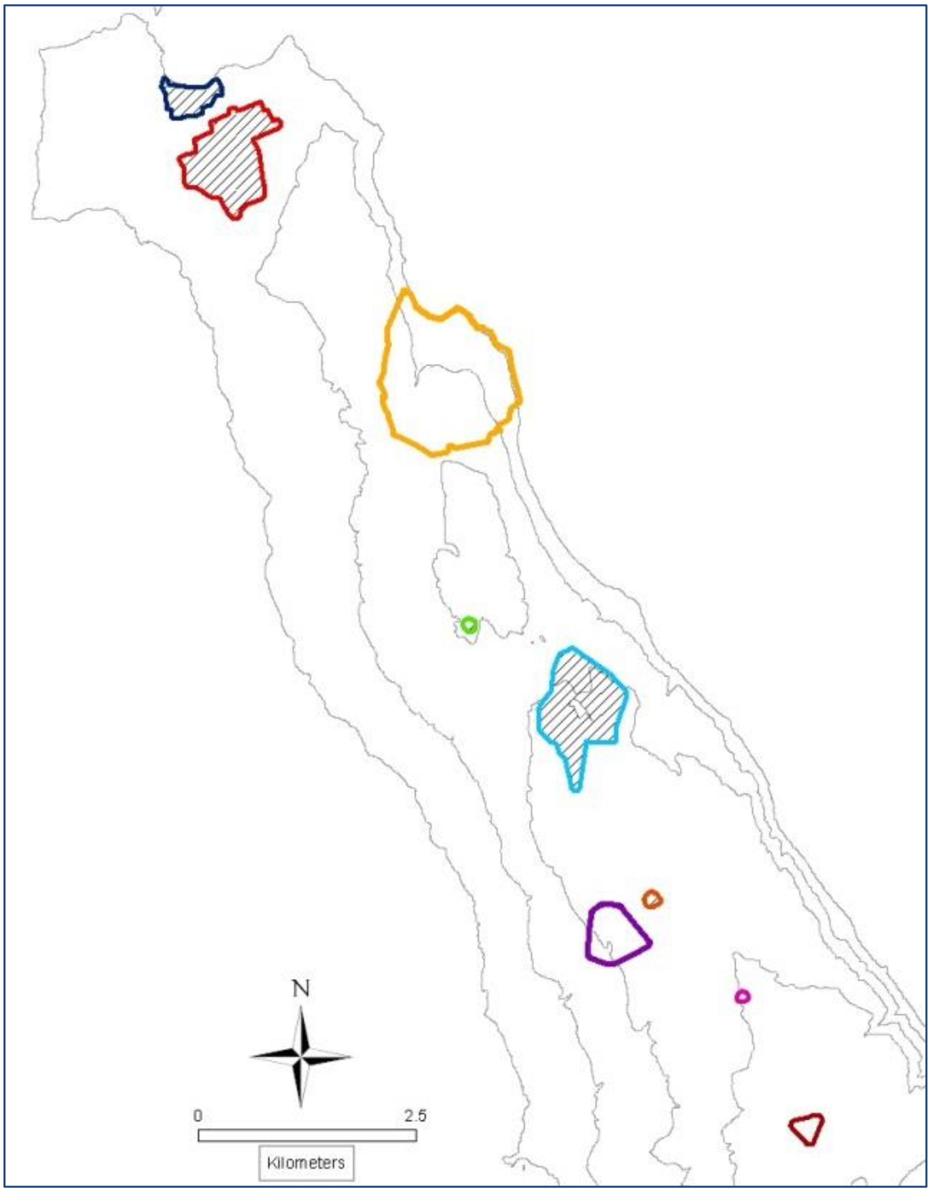

Figure 1. Map of SCI infestations. Known Argentine ant infestations on San Clemente Island as of April 2015. Areas with diagonal lines are sites treated with thiamethoxam bait in 2014. Each infestation is outlined in a different color: dark blue, BUDS; red, Airfield; yellow, Wilson Cove; green, Wall; light blue, Dump; purple, Magazine; orange, VC3; pink, MIR; and brown, Quarry.

Figure 2. Liquid bait. Fully imbibed hygroscopic beads in a mixing container. Beads are loaded into the helicopter hopper using 18.93L (5 gal) buckets pictured here.

Figure 3. Hand deployment of liquid bait. a) Technician deploying bait with a handheld spreader. b) Technician using the ATV spreader to deploy bait.

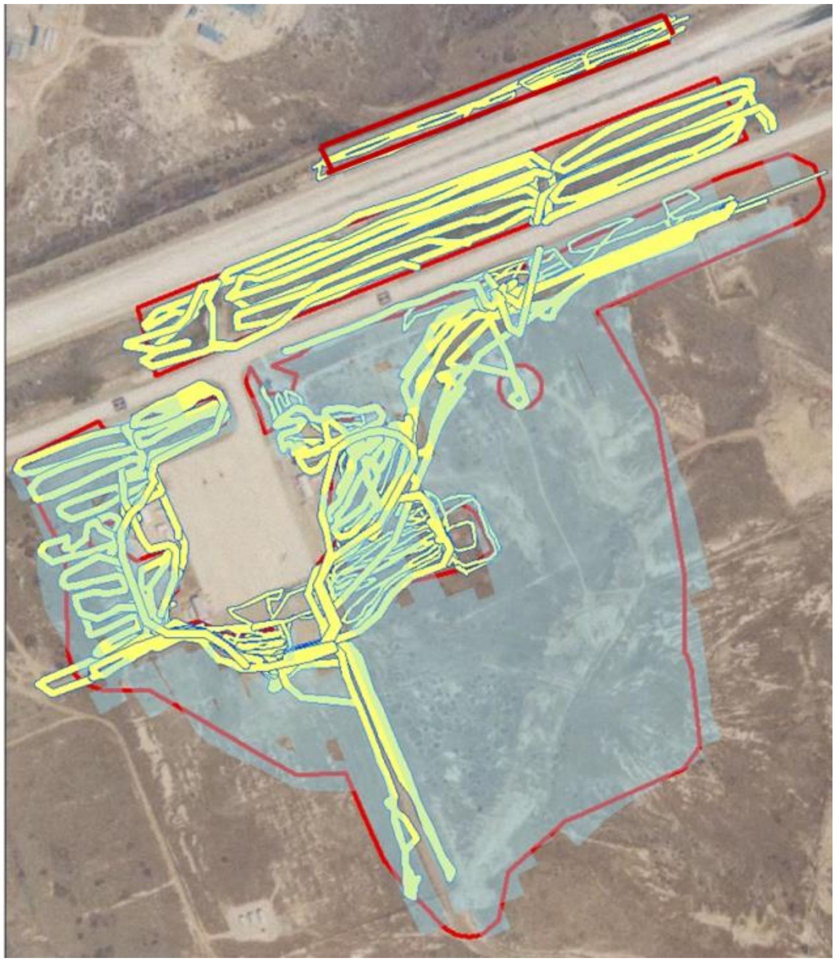

Figure 4. Deployment tracks. Bait deployment tracks at Airfield site for September 2014 deployment. Yellow tracks are hand and ATV deployments, light blue tracks are area covered by helicopter deployment.

Figure 5. Aerial deployment tracks. Aerial coverage of Dump site during the 6 Sep 2014 deployment, each swath is represented in light yellow.

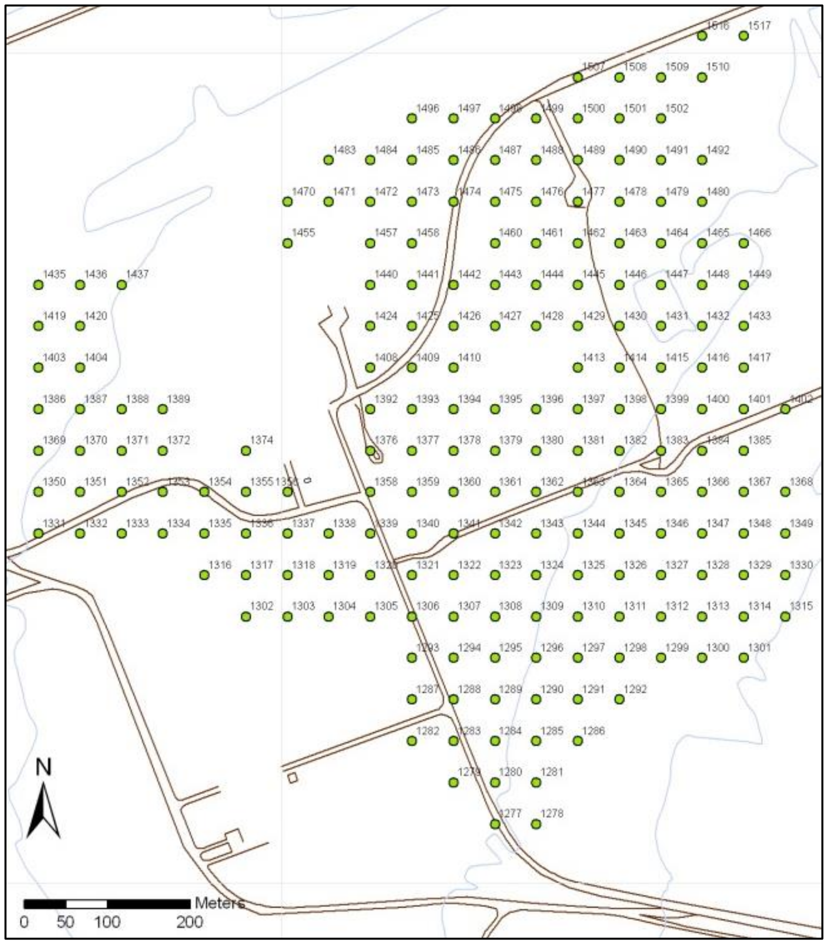

Figure 6. Map of Airfield. Monitoring points at Airfield spaced 50m apart across the infestation.

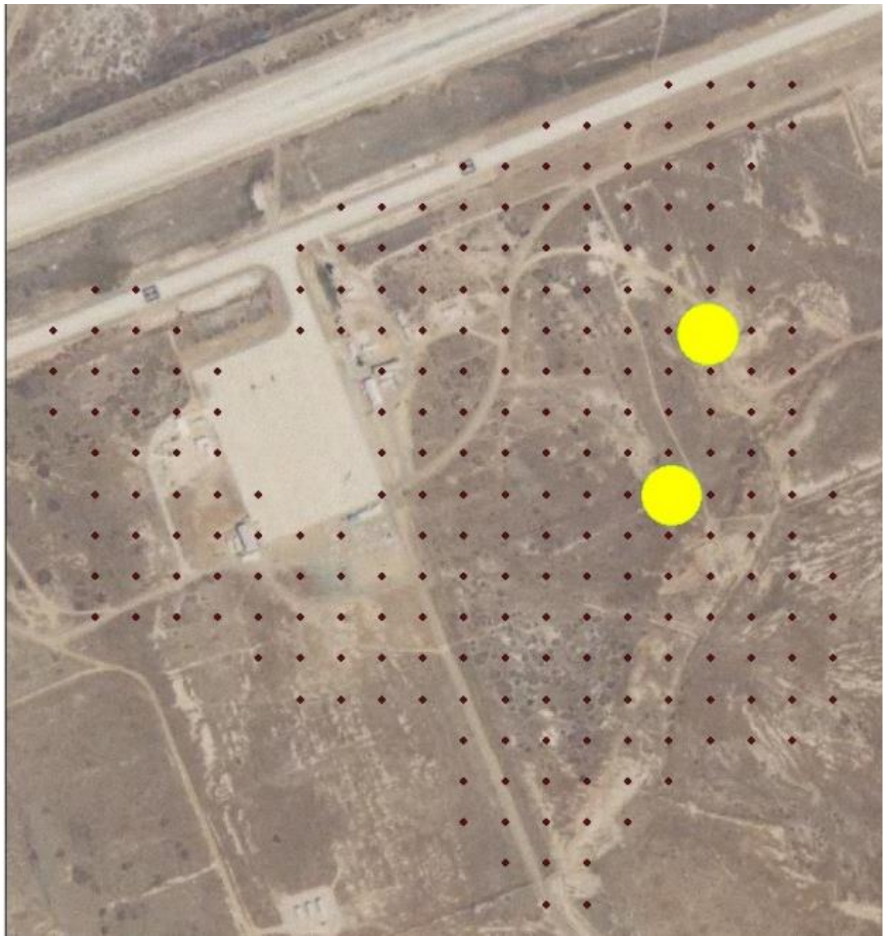

Figure 7. Remnant Argentine ant populations. Points with Argentine ants after seven deployments at Airfield site in yellow, small red dots are monitoring points.

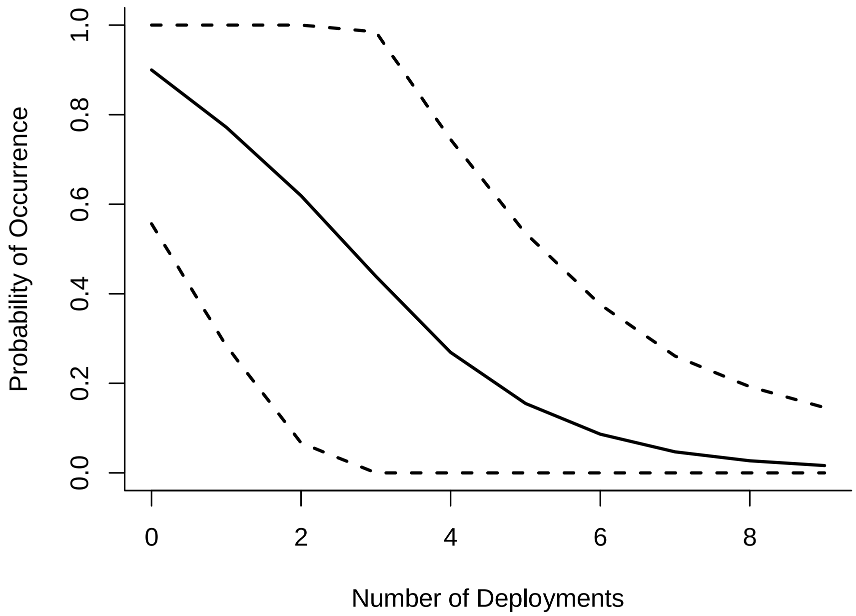

Figure 8. Graph of Argentine ant occurrence. The probability of occurrence (y-axis) after pesticide treatment (x-axis) across all seven sites. Dashed lines indicate 95% credible intervals.

Figure 9. Bait. Argentine ants foraging on bait after a hand deployment at Airfield site.

Tables Chapter II.

Table 1. Deployment and Monitoring schedule. Deployment and monitoring was conducted for April through November 2014. The number of replicates per month is indicated by the number in each cell. Grey shading is monitoring and red shading is pesticide deployment for the respective month.

Treatment & Monitoring Timeline								
Site	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**
Airfield				4	0	1		1
				2*	2	1	2	2
Dump				2	1	1		1
					2	1	2	2
BUDS					1			1
					2	2	1	1
VC3	1	1	1	2	1	1		1
	1*	1*		2	1	1	2	1
Wall			1	2	1	1		1
				2	1	1	2	1
Wilson Cove				2	1	1		1
Magazine				2		1		1

* Experimental treatments in small plots at Airfield and VC3 to test feasibility of hand baiting.

Table 2. Treatment Efficacy at sites treated in 2014. BUDS site was not analyzed since zero ants were detected at monitoring points prior to and after treatment.

Site	***t***	**df**	***p***
Dump	3.34	85	<0.01
Airfield	4.70	168	<0.01
Wall	1.52	41	0.14
VC3	2.22	32	0.03
All Sites	4.54	385	<0.01

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CHAPTER III. Monitoring Argentine ants

Introduction

Native to South America, the Argentine ant *Linepithema humile* (Mayr) has been introduced in several areas with Mediterranean climates, resulting in many negative ecological impacts (Harris *et al.*, 2002; Suarez *et al.*, 2000, 1999). Argentine ants develop large population sizes and are very aggressive toward other arthropods, especially other ants. Such traits may have provided the original colonists with a competitive advantage over native ant species (Harris *et al.*, 2002). Furthermore, Argentine ants alter biological communities by negatively disrupting other invertebrates including pollinators, plants, and terrestrial vertebrates (Hanna *et al.*, 2014; Holway *et al.*, 2002; Lach, 2007; Suarez *et al.*, 2000).

Because of these detrimental impacts of the species' pervasive expansion, vast amounts of resources have been allocated to Argentine ant control in urban and agricultural areas (Rust *et al.*, 2000; Suarez *et al.*, 1999). For ecologically sensitive habitats such as California's Channel Islands (Jenkins *et al.*, 2015), new control efforts are underway to minimize the environmental impact of the pesticide treatments for *L. humile* (Boser *et al.*, 2014; Rust *et al.*, 2015). Recently, 0.000006% thiamethoxam liquid bait was used with a novel delivery method to treat ecologically sensitive habitats on Santa Cruz Island, CA (Boser *et al.* 2014; Rust *et al.* 2015). As Argentine ant eradication efforts are implemented in these natural areas, the need for a standardized detection protocol is essential to minimize the chance of false negatives (i.e., indicating that ants are absent when they are present). Current detection methods, which utilize a combination of visual searching and baiting with sugar water (Hoffmann and O'Connor, 2004; Hoffmann *et al.*, 2010; Ward and Stanley, 2012), can be extremely time consuming and difficult to implement at such large scales as the California's Channel Islands. To increase the likelihood of Argentine ant detection with minimal cost, this study focused on the development of effective bait traps. When properly designed, the bait trap method would allow for improved detection of Argentine ant populations with high sensitivity regardless of their fluctuating activity levels throughout the day (Ward and Stanley. 2012).

In aid of creating effective detection protocols, five bait traps designs were developed and their effectiveness (or sensitivity) in determining the presence of Argentine ant populations was tested. Efficacy was assessed with monthly field trials from June 2013 through November 2014 with an additional monitoring round in February 2015. Bait traps are non-toxic monitoring tools used to monitor or detect ants, usually used for delineating infested areas after ants have been observed. To better understand how to detect small populations of Argentine ants; other environmental co-variables (i.e. soil moisture, vegetation type, and seasonality) that could contribute to the extent of Argentine ant populations were also considered in conjunction with bait trap data. In particular, soil moisture and soil temperature are known to play important roles in the movement of Argentine ants (Markin 1970; Krushelnycky and Reimer 1998; Menke and Holway 2006; Heller *et al.* 2006; Brightwell *et al.*, 2010). Based on the relationships among soil moisture, soil temperature and Argentine ant occurrence in the field, we might be able to predict the current and future locations of Argentine ants.

Materials and Methods

Assessment of Bait Traps.

To determine which monitoring methods are most effective in determining the presence of Argentine ant populations after or during the Argentine ant eradication programs on San Clemente Island, several different bait trap formulations were developed and tested at three sites (Dump, Airfield and Wilson Cove) (Fig. 1). All three sites are in located in disturbed, non-native grassland areas in the northern half of the island. Starting in June 2013, the first site (Dump) was initially used for bait trap assessment until May 2014 by which time numerous bait traps were disturbed or removed from the study by the San Clemente Island Foxes (*Urocyon littoralis clementae*). Subsequently, the second site (Airfield) was added in May 2014 and monitored until August 2014the majority of bait traps were disturbed or removed by foxes. At which point, the third site (Wilson Cove) was added and monitored in October and November 2014 and again in February 2015. Each monitoring plot consisted of a grid of 20 monitoring points spaced 20 m apart over an area of approximately 2800m^2. The bait trap assessments were conducted every month at one plot from June 2013 to November 2014, except in April and May 2014 when either no samples were taken or two plots (Airfield and Dump) were used, respectively.

Between June 2013 and March 2014, the relative abundance of Argentine ants at each monitoring point was estimated by setting pitfall traps (50-ml vials filled ¾ full of a salt and soap water solution) for each monitoring period of the bait trap study. After 24 hours, the pitfalls traps were collected and the number of Argentine ants in each trap was counted to determine the abundance of Argentine ants at that point. After being used 10 times between, pitfall trapping was discontinued because the pitfall traps the pitfall trap counts were significantly skewed by the presence of foraging trails to the particular pitfall traps.

The contents and design of the bait traps were adapted from previous studies (Krushelnycky and Reimer 1996;Greenberg and Klotz 2000 Rust *et al.* 2000; Choe *et al.* 2012) based on cost, availability and ease of use in the field (Table 1). For the liquid attractants, bait traps consisted of 50-ml vials (Celltreat 229420 Polypropylene Centrifuge Tube, Celltreat Scientific Products, Shirley, MA) containing either one large or two small cotton balls (~0.5 g dry weight) soaked in the appropriate liquid solution (~4.0 g). The liquid attractant solutions consisted of one of the following four formulations: sugar water (25% sugar solution), protein powder (Vanilla Gold Standard 100% Casein, Optimum Nutrition Inc., Aurora, IL) ,pheromone 1 [(Z)-9-hexedecenal (0.80g/ml), Bedoukian Research Inc., Danbury, CT] and pheromone 2 [microencapsulated (Z)-9-hexedecenal (0.56g/ml); Suterra, LLC., Bend, OR] (Table 1). A fifth bait trap employed a solid attractant consisting of 50-ml vials containing ~1 g of sugar-egg mixture. All of the bait traps were freshly prepared on the day when they were set in the field to ensure the consistency in the freshness and quality of bait every replication. The bait traps were set in the afternoon, after 1400 hrs but before sunset, in a block design 1-2m apart from each other surrounding the center of a monitoring point and pitfall trap (Fig. 2). After 24h, the bait traps were collected and stored in a freezer until the number of ants in each bait trap was counted.

Varying numbers of bait trap types were used throughout the study as more pheromone combinations were developed (Table 2). The first bait traps combination (i.e. sugar water, sugar-egg and protein powder) was used June 2013. The second combination of bait traps (i.e. sugar water, sugar-egg, protein powder and pheromone 1) was used July and August 2013. The third bait traps combination (i.e. sugar water, sugar-egg and pheromone 1) was used September 2013- May2014. The fourth combination of bait traps (i.e. sugar water, sugar-egg, pheromone 1 and pheromone 2) was used May-November 2014. An additional bait trap trial was conducted in Feb 2015 using sugar water, sugar-egg, and pheromone 2 at the Wilson Cove site.

Efficacy of Bait Traps at Low Densities

From August 2014 to November 2014, the first two sites (Dump and Airfield) were treated seven times with 0.0006% thiamethoxam bait as described in Chapter 2. After the start of the thiamethoxam application these sites were used to test bait trap efficacy when Argentine ant densities were suppressed by treatment. This bait trap efficacy study was conducted six times (two at the Airfield and four at the Dump plots) in July-September 2014. This study was implemented as described above with four baits (i.e. sugar-egg, sugar water, pheromone 1 and pheromone 2) and all of the data was transcribed into presence -absence binary data instead of total ant sums.

Soil Moisture and Temperature.

Starting in June 2014, soil moisture and temperature measurements were collected using a ProCheck GS3 Stereo (Decagon Devices Inc.) soil moisture sensor by inserting the probe near the center of the monitoring point as bait traps were retrieved from the field.

Pheromone Concentrations.

To determine the effect of pheromone concentration in the bait trap performance, varying quantities of pheromone were tested with Argentine ant foraging trails. In this study the microencapsulated pheromone formulation (Suterra, LLC.) was used. Study points were selected by visually searching for active Argentine ant foraging trails in the Wilson Cove site. In particular, each point had a foraging trail leading to the sub-shrub species, *Acmispon dendroideous* var. *traskiae* (Fabaceae); and all had similar annual vegetation, vegetation coverage and topography. The study points were marked with blue pin flags.

Once a study point was established, the activity of Argentine ants was estimated by counting the number that crossed a fixed point on an *Acmispon* branch for 15 s. Sugar water containing different amounts (0, 0.15, 1.0, 5.0 and 10.0 ml into 3.79 L of 25% (w/v) sugar water) of microencapsulated formulation (Z)-9-hexadecenal (Suterra, LLC.) were tested (Table 3). Cotton balls (one large cotton ball or two small cotton balls per vial) were soaked in one of the pheromone solutions, placed in 50 mL vial (Celltreat) and capped. Bait traps were placed flush to the ground, approximately 0.5m away from the established foraging trail, usually positioned in the same cardinal direction. In some instances, grass was removed to ensure accessibility to the bait traps. The bait traps were monitored for presence or absence twice: 30 min and 1 h after being placed. After 2 h, the bait traps were retrieved and frozen and the total number of ants captured in the bait traps was counted. Each concentration of the pheromone was tested at one point for a total of 20 points per sampling period. A total of 140 study points were used.

Statistical Analysis.

All data analysis was analyzed with the program R v.3.1.2 (The R Foundation for Statistical Computing, 2014). Bait Trap Assessment results were grouped by the bait trap combination and analyzed with a post-hoc analysis using the lmer function in the "lme4" package (Bates *et al.* 2014) with outliers removed (2.5 SD trim); points were set as random effects whereas date, bait and date*bait interaction were set as fixed effects. Low-density bait trap presence-absence data from Airfield and Dump sites (July-Sept 2014), intensive monitoring grids experiment, and pheromone concentration experiment were analyzed with Pearson's Chi-square distribution test in the "stats" package (R Core Team and contributors worldwide, 2014).

Soil moisture and temperature correlations to Argentine ant activity were analyzed with Pearson's Correlation Coefficient using the cor function in the "stats" package and rcorr in the "Hmisc" package (Harrell 2014).

Results

Assessment of Bait Traps.

With all bait trap experiments, except the first trial (June 2013), date and bait trap type had a significant interaction on average Argentine ant activity (ΔAIC=66, X^2=135.41, df =35, p <0.01). In the first bait trap trial (June 2013), protein powder captured significantly more ant activity than sugar water (t=5.07, df= 58, p <0.01) and sugar-egg (t=4.71, df =58, p <0.01), which did not differ significantly (t=-0.26, df =58, p

=0.78). For the second trial (July-August 2013) pheromone 1 bait trap was added to the study. Sugar water was significantly less attractive than protein powder (t=-3.80, df=109, p <0.01) and pheromone1 (t=-3.57, df=109, p <0.01) but not to sugar-egg (t=0.547, df=109, p <0.59) in July and August 2013 (Fig. 3). The protein powder bait trap was removed from trials starting October 2013 due to the susceptibility of the bait trap to become moldy. Bait traps used in the fourth set of trials (October 2013-May 2014) were sugar water, sugar-egg and pheromone 1. Sugar water bait traps were significantly different than pheromone1 (t=-4.48, Dendf=345, p<0.01) but not different from sugar-egg (t=0.63, Dendf=345, p=0.53) throughout this trial. However, bait trap efficacy did alter seasonally (Fig. 3). In October, November and December 2013, sugar water baits had less ant activity than pheromone 1 and sugar-egg (Table 3). However in January through May 2014, there is no significant difference between pheromone 1 and sugar water (Table 3). Starting in January 2014, there is a trend of increased ant activity at sugar-egg bait traps over the other traps. Sugar water bait traps are significantly different than sugar-egg (Table 4). This trend continued until May 2014 and is the first instance of sugar-egg having the lowest ant activity. For the second monitoring round in May 2014 and afterwards, the pheromone 2 (microencapsulated) bait trap was added to the study.

The addition of the second pheromone trap resulted in no overall difference between bait traps from May 2014-November 2014 (ΔAIC=4, X^2=1.95, df =3, p=0.58). However in July and October there is a significant difference between sugar water and sugar-egg bait traps (t=3.75, Dendf=260.5, p<0.01, and t=2.78, Dendf=258.76, p<0.01, respectively). During the last trials (May 2014-Feb 2015), if activity at pheromone 1 and pheromone 2 bait traps are combined for data analysis, there is a significant bait trap preference and seasonality affect (ΔAIC=13.44, X^2=33.44, df =10, p<0.01).

Low-Density Efficacy of Bait Trap Designs.

The two pheromone solutions were equally effective in detecting the presence of Argentine ants during low-density monitoring (X^2=0.02, df =1, p =0.89). To understand the dynamics between bait traps, ant activity from both pheromone traps were pooled together into "pheromone" for analysis of bait trap preference. When Argentine ant numbers are relatively low after treatment (July - September 2014), bait traps with pheromone had a higher detection rate when compared to sugar water (X^2 =5.90, df =1, p =0.02). The sugar-egg bait traps had an intermediate level of effectiveness in detecting Argentine ants, not being statistically different from sugar water and pheromone bait traps (X^2 =2.88, df =1, p =0.09) (Fig. 4).

Soil Moisture and Temperature.

During monitoring periods February 2014, June 2014 through November 2014 and February 2015, soil temperature was a significant fixed-effect (ΔAIC=207.42, X^2=209.42, df=1, p< 0.01) but soil moisture was not significant in determining Argentine ant activity and bait trap preference (ΔAIC=1.4, X^2=0.56, df=1, p =0.45). The correlation (Pearson's Correlation Coefficient, r) between soil temperature and Argentine ant activity at each site was weakly negative (Fig. 5): Airfield (n= 121, r =0.16, p =0.09), Dump (n =58, r =0.25, p =0.06) and Wilson Cove (n= 173, r =-0.15, p =0.05).

Pheromone Concentration.

There was no statistical difference in Argentine ant capture rate between different pheromone concentrations and sugar water after 0.5h (X^2=0.41, df =1, p =0.26), 1h (X^2=2.39, df =1, p =0.06), or 2h (X^2=0.35, df =1, p =0.55).

Conclusions

Changes in dietary requirements are known to influence the attractiveness and thus effectiveness of the bait matrix for Argentine ants to some degree; protein is typically more effective in the spring as brood size increases while carbohydrates are preferred in late fall or winter as nest size shrink (Krushelnycky and Reimer 1998; Rust *et al.* 2000). The first year of this study supports this seasonality effect with higher Argentine ant activity captured at carbohydrate bait traps (pheromone) in the late fall while more Argentine ants were captured at protein bait traps (sugar-egg or protein powder) on average in late spring.

It is interesting that the seasonal trend did not continue into the second year of the study on San Clemente Island. The lack of seasonal change in bait trap preference throughout the year could be driven by the ongoing drought on San Clemente Island in 2014-15. For example, in a 20 year review of the Jasper Ridge Argentine ant infestation in northern California, Gordon and Heller (2014) reported that the drought coupled with interactions with the winter harvester ant have limited the spread of Argentine ants into natural areas *i.e.* areas less influenced by human disturbance. This is likely due to the lack water available away from structures in arid environments (Menke and Holway 2006; Gordon and Heller 2014). Multiple studies have found positive correlations with ant activity and water availability (Krushelnycky and Reimer 1996; Menke and Holway 2006; Enzman *et al.* 2012). Surprisingly, soil moisture (ranging 0.05-0.22 m^3/m^3 across sites and months) was not a significant predictor of Argentine ant activity under the drought conditions experienced during this study. However ant activity did significantly change with soil temperature (ranging from 32.3^0C to 16.5^0C); as temperatures increased, ant activity decreased. Here we show that foraging activity is influenced by soil temperature during dry conditions and all bait traps tested were approximately equally attractive since. This suggests that bait choice may be less important in times of severe water limitation.

Sugar-egg and pheromone were equally effective at capturing ants during the low-density bait monitoring. When monitoring low-density Argentine ant infestations, the addition of the Argentine ant trail pheromone, (Z)-9-hexedecenal, to 25% sugar water increases the likelihood that Argentine ants will be detected compared to sugar water alone. Further research is needed to establish the optimal pheromone concentration to use along with ideal baiting density in monitoring grid. Sugar-egg bait traps are an effective monitoring bait trap but were more susceptible to disturbance or removal by Island Foxes overnight (19.3% removed) compared to pheromone (8.3%) and sugar water (4.39%) bait traps during the low density monitoring study. If animal disturbance is a potential concern during the eradication project, then our data suggests pheromone or sugar water would be the more effective bait trap as they are less likely to be disturbed by curious small mammals.

When choosing monitoring methods, land managers should keep in mind the following: (1) the season during which monitoring is to occur and how this plays into dietary needs of the colony, (2) soil temperature, (3) bait trap disturbance potential and (4) relative infestation density (high or low). The use of bait traps as a monitoring tool has the potential to be more cost effective than the current method of intensive visual searching plus baiting. If baits trap locations are mapped with handheld GPS units during retrieval, this will maximize the monitoring efforts by mapping both remnant Argentine ant populations and areas of no detection. The recommendations emerging from this research should be used by land managers to increase their confidence in Argentine ant detection results, especially when implementing landscape-scale eradication efforts.

Figures Chapter III

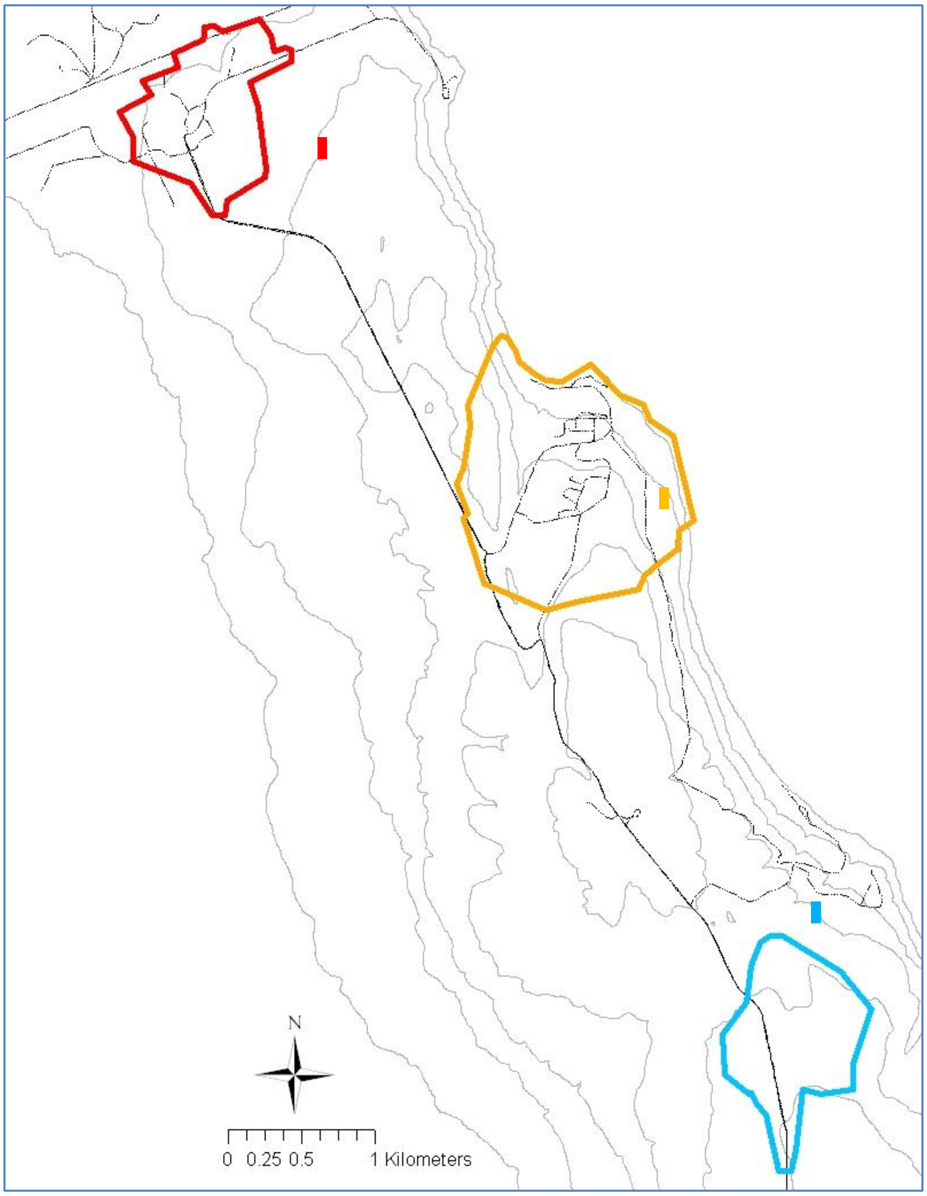

Figure 3. Bait trap assessment sites on SCI. Rectangles represent grids within each site Airfield (red outline), Wilson Cove (yellow outline) and Dump (blue outline).

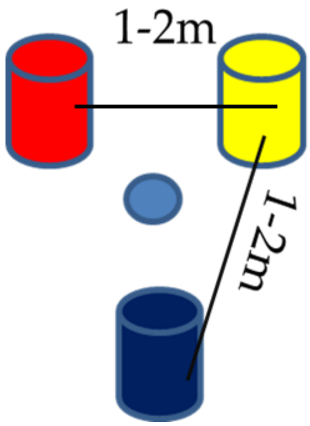

Figure 24. Schematic of bait traps surrounding a monitoring point (blue circle) placed 1-2m apart in similar microhabitat.

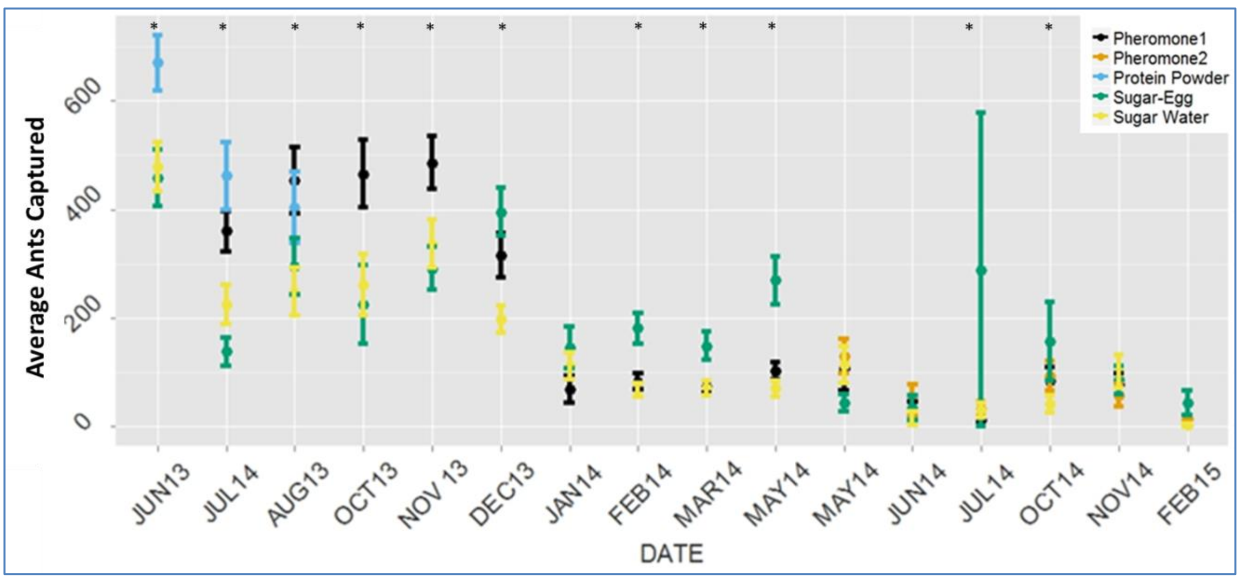

Figure 35. Assessment of bait trap designs. Mean number of Argentine ants collected by the five different bait traps per monitoring round (y-axis) over the extent of the study period (x-axis). Bars represent standard error (SE). Bait trap designs are represented by different colors: Pheromone 1, black; Pheromone 2, orange; Protein Powder, blue; Sugar-Egg, green; and Sugar Water yellow. * Represent bait traps significantly different (p<0.05) from Sugar Water bait traps.

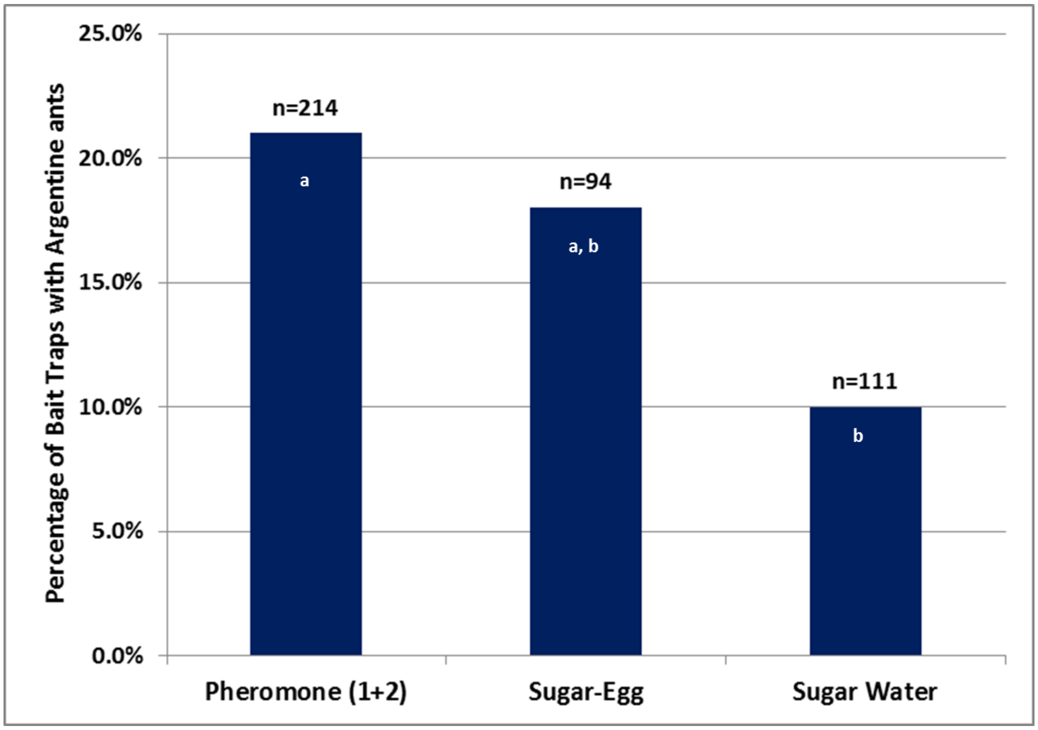

Figure 46. Low-density bait trap assessment. The percentage of bait traps with Argentine ants captured during low density monitoring from July-September 2014. Letters refer to bait traps that were significantly different ($p<0.05$) from each other.

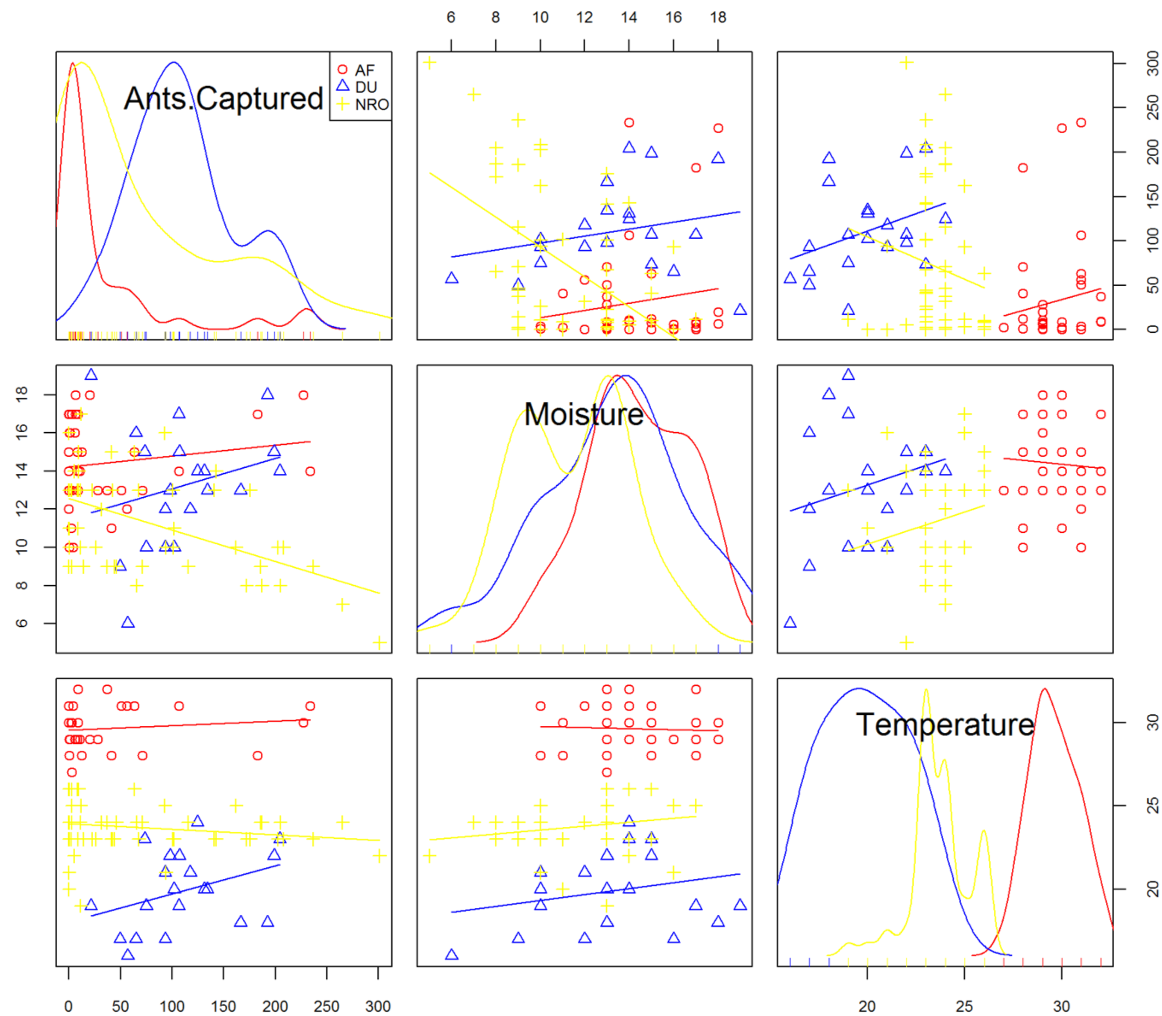

Figure 57. Ant activity scatterplot. Correlation of average Argentine ant captured at bait traps to soil temperature and soil moisture over six months at three sites: Airfield, red; Wilson Cove, yellow; and Dump, blue.

Tables Chapter III

Table 1. Bait traps attractants.

Name	Description
Sugar Water	25 % sucrose water (900 g of sugar to 3.79 L of water) soaked cotton ball
Protein Powder	Vanilla Gold Standard 100% Casein protein powder (160 g) mixed in 25% sucrose water (3.79L) soaked cotton ball
Pheromone 1	Argentine ant pheromone (0.05 ml (Z)-9-hexedecenal dissolved in 5mL of 95 % ethanol mixed in 2.5L of 25 % sucrose water) soaked cotton ball
Pheromone 2	Argentine ant pheromone (0.15mL microencapsulated (Z)-9-hexedecenal mixed in 2.5L of 25 % water sucrose solution) soaked cotton ball
Sugar-Egg	Scrambled egg sucrose mixture (2 eggs cooked with 66.67g sugar in a cast iron skillet)

Table 2. Dates bait traps were deployed. The first six highlighted columns (dark grey) represent six months from June-December 2013; the next ten columns (light grey) represent months from January-November, 2014; and the last (pink) represents February 2015. Sugar water (blue) and sugar-egg (orange) bait traps were used for the entire study, pheromone 1 (yellow) was used from October 2014-October 2015, pheromone 2 (red) was used from June 2014 to February 2015, and protein powder (purple) was used in June and July 2013.

	Dates Deployed																
Bait Trap	J	J	A	O	N	D	J	F	M	M	J	J	A	S	O	N	F
Sugar Water																	
Sugar-Egg																	
Pheromone 1																	
Pheromone 2																	
Protein Powder																	

Table 3. Pheromone Concentrations. All pheromone concentrations were mixed in 25% sugar water.

Name	Emulsified Pheromone (ml)	Actual Pheromone (%wt*ml)	Pheromone Concentration (ml/l)
Sugar	0	0	0
0.15	0.0396	2.22E-04	2.22E-07
1	0.2639	0.0015	1.48E-06
5	1.3193	0.0074	7.39E-06
10	2.6385	0.0148	1.48E-05

Table 4. Bait traps assessment from June 2013-November 2014.

Trial	Date	Bait	Estimate	Std. Error	Den. df	t value	Pr(>|t|)
Trial 1	Jun-13	protein*	269.19	53.05	58.92	5.075	4.16E-06
	Jun-13	sugar-egg	13.91	54.19	59.19	0.257	0.798
Trial 2	Jul-13	pheromone 1*	133.16	63.68	105.69	2.091	0.03893
	Jul-13	protein*	200.52	64.64	106.32	3.102	0.00246
	Jul-13	sugar-egg	-88.73	63.68	105.69	-1.393	0.16645
	Aug-13	pheromone 1*	209.78	67.68	105.76	3.1	0.00248
	Aug-13	protein*	154.63	66.52	105.07	2.324	0.02203
	Aug-13	sugar-egg	46.44	66.52	105.07	0.698	0.48669
Trial 3	Oct-13	pheromone 1*	253.051	56.525	349	4.477	1.03E-05
	Oct-13	sugar-egg	-35.337	56.497	347	-0.625	0.532068
	Nov-13	pheromone 1*	249.335	53.325	345.7	4.676	4.21E-06
	Nov-13	sugar-egg	-45.684	52.588	345	-0.869	0.385605
	Dec-13	pheromone 1*	118.59	59.38	348.5	1.997	0.046582
	Dec-13	sugar-egg*	196.95	58.39	352.2	3.373	0.000826
	Jan-14	pheromone 1	-43.72	51.94	345.7	-0.842	0.400524
	Jan-14	sugar-egg	34.11	52.59	345	0.649	0.51707
	Feb-14	pheromone 1	140.9	51.26	345	0.291	0.77
	Feb-14	sugar-egg*	113.05	51.26	345	2.206	0.028
	Mar-14	pheromone 1	2.3596	52.6828	364.4	0.045	0.964301
	Mar-14	sugar-egg	78.1096	52.6828	346.4	1.483	0.139
	May-14	pheromone 1	31.55	51.2565	345	0.616	0.53861
	May-14	sugar-egg*	224.45	51.2565	345	4.379	1.58E-05
Trial 4	May-14	pheromone 1	-37.153	26.47	253.8	-1.404	0.16167
	May-14	pheromone 2	15.05	26.101	253.56	0.577	0.564711
	May-14	sugar-egg	-61.852	26.877	254.08	-2.301	0.022185
	Jun-14	pheromone 1	17.017	30.379	256.4	0.56	0.57868
	Jun-14	pheromone 2	16.59	30.364	256.2	0.547	0.585195
	Jun-14	sugar-egg	14.2	31.169	256.3	0.456	0.64907
	Jul-14	pheromone 1	-8.207	30.912	255.4	-0.265	0.79085
	Jul-14	pheromone 2	11.96	29.319	254.19	0.408	0.683678
	Jul-14	sugar-egg*	240.379	64.174	260.52	3.746	0.000221
	Oct-14	pheromone 1	34.2	29.56	259.03	1.157	0.24847
	Oct-14	pheromone 2	45.97	28.84	260.51	1.594	0.112206
	Oct-14	sugar-egg*	98.29	35.35	258.76	2.781	0.005824
	Nov-14	pheromone 1	-24.729	29.497	256.95	-0.838	0.402
	Nov-14	pheromone 2	-44.29	29.354	254.85	-1.509	0.132575
	Nov-14	sugar-egg	-7.689	31.543	256.19	-0.244	0.8076

* values that are statistically significant from sugar water bait traps.

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CHAPTER IV. Conclusions

Management efforts implemented on California's Channel Islands to eradicate the Argentine ants have successfully reduced this invasive species in ecological sensitive areas (Boser *et al.* 2014; Rust *et al.* 2015; this study). As shown here, treatment of Argentine ant at five sites on San Clemente Island was effective with a 99.86% reduction in ant activity over the course of a few months. Since the Channel Islands are listed as one of the nine areas for immediate conservation attention in the United States (Jenkins *et al.*, 2015), continued efforts are imperative to fully eradicate Argentine ants from the archipelago.

The methods used in this study and on Santa Cruz Island (Boser *et al.* 2014; Rust *et al.* 2015) have the potential to be implemented across a wide range of ecologically sensitive areas as well as for the management of a variety of invasive species. This type of toxicant baiting and monitoring design will be most effective for species that prefer sugary liquids or sugary protein and a variety of bait trap attractants should be considered for each invasive species. Thus, land managers should use the protocols here as the framework for developing eradication programs allowing for the particular idiosyncrasies of the landscape and species to be treated.

Because the polyacrylamide hygroscopic beads are relatively inexpensive (approximately $11-$15/kg) and easy to apply, the liquid baiting method with the polyacrylamide matrix would be an affordable option if large areas are to be treated with a purpose of full eradication rather than repeated management programs to achieve the population size under certain threshold level (Rust *et al.* 2015). Future research should focus on developing biodegradable hygroscopic beads; as a limitation to the methods mentioned in this study is that the hygroscopic beads are not readily biodegradable in field conditions (Rust *et al.* 2015). Moreover, given its importance and relative attractiveness to Argentine ants, additional studies should investigate how protein could be incorporated into the hygroscopic beads. Foraging Argentine ants will readily consume protein baits during the peak season of colony reproduction, and a proteinaceous bait should be considered if the baiting is planned in the spring (Baker *et al.*, 1985; Krushelnycky and Reimer, 1998; Rust *et al.*, 2000). This would also allow the possible application of the hygroscopic bead matrix to baiting other species of ants that would prefer proteinaceous bait materials to a sugary liquid (e.g. *Solenopsis invica*, *Pheidole megacephala* and *Wasmannia auropunctata*).

To maximize the likelihood of a successful eradication program and to minimize the chance of false negatives while monitoring low-density sites, it is imperative to have optimal detection methods (Hoffmann *et al.*, 2010). We are only now recognizing the need for more detection probability assessments in conservation efforts to fully understand invertebrate population fluctuations, especially in areas where control or eradication programs are implemented.

As the eradication projects of Argentine ants on the Channel Islands are approaching the pivotal moment of transitioning from the treatment to the post-treatment monitoring, it is important to use the most effective monitoring protocols to detect remnant individuals as even small populations of Argentine ants can be reproductively prolific (Hoffmann, 2011; Holway *et al.*, 2002; Tsutsui and Suarez, 2003). This study demonstrates that the bait traps sugar-egg and pheromone are both effective at detecting Argentine ants. Thus when monitoring low-density Argentine ant infestations, the addition of the synthetic Argentine ant pheromone, (Z)-9-hexedecenal, to 25% sucrose water increases the likelihood that Argentine ants will be detected compared to the sucrose water alone. Bait traps with sugar-egg were highly susceptible to disturbance by Island Foxes. To avoid such disruption, larger bait stations will be used to limit the foxes' capability to remove the bait traps from the original locations.

Fluctuations of Argentine ant activity over the course of a year and a half show the importance flexible bait or monitoring protocols based life history traits of the target species. On San Clemente Island bait traps with the synthetic Argentine ant pheromone were most attractive in the late summer and fall while the protein bait traps (sugar-egg and protein powder) were most attractive in the spring to mid-summer. During the continued drought conditions on SCI (2014), bait traps captured similar number of ants at lower abundances. With increasing soil temperatures Argentine ant activity at bait traps decreased however sugar-egg bait traps captured more ants during this period than the other bait trap attractants. This information is essential when considering what monitoring protocols to use for an Argentine ant eradication projects.

To increase detection confidence for Argentine ant management practices land managers should keep in mind the following: (1) the seasonality effects of foraging, (2) abiotic factors (soil temperature and soil moisture), (3) potential bait trap disturbance, and (4) relative infestation density (high or low).

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