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ARGENTINE ANT MANAGEMENT IN CONSERVATION AREAS: RESULTS OF A PILOT STUDY

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ABSTRACT.—Argentine ants (*Linepithema humile*) have invaded many areas of conservation concern, including half of the California Channel Islands. On Santa Cruz Island, the species has invaded approximately 2% of the island, and the infestations are expanding. Argentine ants displace many other invertebrates, and their expansion throughout the island could lead to the extirpation of native invertebrate species and the disruption of key ecological processes (e.g., plant-pollinator interactions and seed dispersal). We describe a treatment protocol to manage or eliminate Argentine ants on Santa Cruz Island developed by The Nature Conservancy and the National Park Service, in collaboration with academic and pest control specialists. We combined low-concentration toxicant baits with efficient dispersal methods to treat landscape-scale Argentine ant infestations in rugged terrain and dense vegetation with minimal impact to nontarget species. From May to October 2012, we applied our baiting protocol within 2 study sites, totaling 7.8 ha on Santa Cruz Island. In May 2013, one year post treatment, we observed >99% reduction in Argentine ant activity in treatment plots compared to untreated plots, using 2 different monitoring techniques. While further testing and monitoring is needed, these results suggest this protocol may be an effective tool to eliminate Argentine ant infestations from this type of habitat and terrain.

RESUMEN.—Las hormigas argentinas (*Linepithema humile*) han invadido muchas áreas de interés para la conservación, incluyendo la mitad de las Islas del Canal de California. En la Isla Santa Cruz, la especie han invadido aproximadamente un 2% de la isla y la infestación se está expandiendo. Las hormigas argentinas desplazan a muchos otros invertebrados, y su expansión por toda la isla podría llevar a la eliminación de especies invertebradas nativas y a la interrupción de procesos ecológicos clave (es decir, interacciones planta-polinizador, dispersión de semillas). Describimos un protocolo de tratamiento para eliminar a las hormigas argentinas de la Isla Santa Cruz desarrollado por The Nature Conservancy y el servicio del Parque Nacional, en colaboración con académicos y especialistas en control de plagas. Combinamos cebos de baja concentración tóxica con métodos de dispersión eficaces para tratar a las plagas de hormiga argentina a escala de paisaje en terrenos escabrosos y vegetación densa con un impacto mínimo en las especies que no estamos intentando eliminar. Desde mayo hasta octubre del 2012, aplicamos nuestro protocolo de cebos dentro de dos lugares de estudio, ocupando un total de 7.8 ha en la Isla Santa Cruz. En mayo de 2013, un año posterior al tratamiento, observamos la reducción >99% de la actividad de la hormiga argentina en los terrenos de tratamiento en comparación con los terrenos sin tratar, usando dos técnicas de monitoreo diferentes. Aún cuando necesitamos más pruebas y monitoreo, estos resultados sugieren que este protocolo puede ser una herramienta efectiva para eliminar las plagas de hormigas argentinas en este tipo de hábitat y terreno.

Argentine ants (*Linepithema humile*) were introduced to California in 1907 and now occupy vast stretches of land along the coast from northern Baja California, Mexico, to the San Francisco Bay area and inland to the Central Valley of California (Suarez et al. 2001). Argentine ants occur on half of California's Channel Islands: Santa Cruz, San Nicolas, Santa Catalina, and San Clemente islands. These islands are recognized for their conservation value, and consequently, the presence of invasive and ecologically harmful Argentine ants is of great management concern. Here, we discuss our efforts to develop methods to eradicate Argentine ants from these conservation areas.

Argentine ants are invasive in agricultural lands, residential areas, and natural habitats where they dominate the native invertebrate fauna and significantly reduce or eliminate native ant populations. The suppression and loss of native ants can have direct and indirect impacts on other invertebrates, plants, and some vertebrates (Ward 1987, Holway et. al. 2002, Suarez and Case 2002, Krushelnycky and Gillespie 2008). As Argentine ants eliminate or compete with native invertebrates within natural systems, plant-pollinator mutualisms

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may be disrupted, affecting native plant fitness by reducing cross-pollination and seed set. The ecological impacts of Argentine ants is exacerbated when in their introduced range genetically similar neighboring colonies show little or no aggression between nests and form huge supercolonies with significantly higher population densities than would otherwise be possible (Suarez et al. 1999).

Argentine ants may be especially destructive in conservation areas intended to protect rare or endangered species and natural ecosystem function. These ants have established in many Mediterranean-type climates around the world: areas which are considered biodiversity hotspots and have disproportionally more to lose to Argentine ant invasions. For example, on Santa Cruz Island, California, native ant diversity has declined from an average of 8 species to only 1.5 species in Argentine ant–invaded areas compared with no decline in uninvaded paired sites (Holway and Hanna 2011). Additionally, Argentine ants on Santa Cruz Island reduce pollination of a native plant by aggressively interfering with visitation of native pollinators and failing to effectively pollinate the plant themselves (Hanna et al. 2015).

Land managers and pest control agencies have attempted to control or eradicate Argentine ant populations in a number of ecologically significant locations around the world including Hawaii (Krushelnycky et al. 2005, 2011), California (Choe et al. 2010), Australia (Hoffmann et al. 2012), and New Zealand (C. Green, NZ Department of Conservation, personal communication). Eradication of large infestations of Argentine ants has proven to be difficult, in part due to their flexible social structure. Colonies consist of multiple queens but can survive if a single queen and a few workers remain (Hee et al. 2000, Tsutsui and Suarez 2003). Reproductive queens are rarely above ground but are fed by worker ants (Markin 1970). Thus, the best method of accessing queens with a toxicant is by distributing it throughout the infested area in bait that is attractive but not immediately lethal to workers.

Developing an eradication protocol for conservation areas is challenging because much of what is known about Argentine ant foraging comes from work in urban and agricultural areas where Argentine ant behavior and resource availability may differ in important ways from wildland settings (Holway and Case 2000). In urban areas, food resources are typically consistently placed and plentiful. Ants establish trails directly to resources and therefore may only encounter bait if it is placed directly on those foraging trails. In wildland areas, resources are often scarce, ephemeral, and patchily distributed. With the exception of some very high-quality natural habitats, ants may employ random-walk foraging to increase the likelihood of encountering food sources (Turchin 1998). In contrast to control protocols in urban environments which usually target ant trails in natural areas, scattered bait may be more successful in wildland areas.

We sought to create an Argentine ant eradication protocol for conservation areas that encompassed 3 key elements: a toxicant, an attractant, and a bait deployment method, all designed to target ant queens. In collaboration with M. Rust (2010), we researched the efficacy of low-concentration toxicants which must be metabolized prior to affecting the organism, allowing time for the worker to feed queens through trophallaxis. We hypothesized that the attractant in the bait must be highly appealing to induce a switch in feeding from natural resources to newly introduced bait. Field testing informed by a literature search revealed that Argentine ants are most attracted to sugar water, tuna, and sugared eggs (Baker et al. 1985, Davis et al. 1993, Hanna and Boser 2012). Additionally, Argentine ants can more easily handle liquid baits (Silverman and Roulston 2001). Thus, we only considered attractants with gel or liquid consistency. Lastly, we needed to distribute the bait so that it would be accessible to all nests within an infestation area, which means deploying bait at least every 4 m^2 (C. Green personal communication). Commercially manufactured baits and existing distribution methods do not meet our desired specifications. Therefore, we created a novel bait: a low-concentration liquid toxicant with a liquid sucrose attractant. We deployed that bait in polyacrylamide pieces, which absorb water and water-soluble chemicals such as toxicants and sugar. The small hydrated polyacrylamide pieces can be easily distributed with consistent coverage.

In May–October 2012, The Nature Conservancy (TNC) and the National Park Service (NPS) tested our new protocol on 2 of the infested areas on Santa Cruz Island. The objective of these trials was to test efficacy and feasibility of the protocol on landscape-scale infestations in common vegetation types of the California Channel Islands (e.g., oak woodland and coastal sage scrub). Specifically, we addressed the following questions: Does the protocol significantly reduce or eliminate Argentine ant activity? How long do Argentine ants feed on polyacrylamide baits? Is there evidence that the protocol may substantially reduce the abundance or activity of nontarget ant species and other invertebrates? Does Argentine ant bait-attractant preference change seasonally?

METHODS

Study Site

Santa Cruz Island is a 250-km^2 ecological preserve located off the coast of southern California approximately 40 km from Santa Barbara. Three quarters of the island is owned by The Nature Conservancy (TNC) and the remainder is owned by the National Park Service (NPS). The island experiences a Mediterranean-type climate of mild, wet winters and warm, dry summers. Island vegetation is predominately coastal sage scrub (~43%), chaparral (~21%), and annual grasslands (~20%) intermixed with areas of oak woodland, pine forest, and riparian corridors. Average yearly precipitation in the island's central valley between 1992 and 2012 was 42 cm. The average daily high and low air temperatures between May and October in 2012 were 25.8 °C and 13.1 °C, respectively. Between May 2011 and April 2012 the central valley received 57 cm of precipitation, and between May 2012 and April 2013 it received 24 cm.

Argentine ants were first recorded in 1996 in 2 areas previously occupied by a contractor of the U.S. Navy. The infestations were delimited in 1999 and in 2010; and based on the estimated rate of spread (10–40 m per year) in that interval, Argentine ants could have arrived as early as the 1960s (Boser 2011, unpublished report). By 2009, four Argentine ant infestations were observed.

In 2012, experiments were conducted at 3 of the 4 Argentine ant infestation sites (Fig. 1): the Field Station (25 ha), Navy Blue site (16 ha), and Valley Anchorage (364 ha). The Field Station infestation is located around the University of California, Santa Barbara field station in the island's central valley and straddles the main drainage on the island. The site is owned by TNC. The waterway in this area is dry 9–12 months out of the year and is mostly open, with occasional clumps of mule-fat (*Baccharis salicifolia*) along with alluvial fan scrub vegetation. The banks of the waterway host stands of mature coastal live oak (*Quercus agrifolia*), island scrub oak (*Quercus pacifica*), and associated coastal sage scrub vegetation, some annual grasslands, and an allee of *Eucalyptus globulus* along the main access road. The Navy Blue site, an abandoned navy antenna site owned by NPS, lies in a transition zone between scrub oak–dominated vegetation and coastal sage scrub and coastal bluff vegetation. There are also sections of rocky outcrops and cliffs with sparse plant cover. The infestation is divided between relatively flat areas to the north and very steep cliffs on the southern edge of the site. The Valley Anchorage infestation is the largest and encompasses coastal bluffs, hills, and canyons on the south side of the island. The infestation occurs on properties owned by TNC and NPS. South-facing hills are covered with coastal sage scrub and coastal bluff vegetation. North-facing slopes are covered with chaparral shrubs. Canyons and drainages contain oaks and mule-fat/willow alliances.

Argentine ants represent an island-wide threat to the biodiversity and conservation values of Santa Cruz Island. The 33 species of native ants on the island, as well as some plant and animal communities, may be adversely affected by Argentine ants (Hanna et al. 2015). Native plant communities are stressed after nearly 150 years of grazing by introduced (now eradicated) ungulates and the introduction of over 170 weed species (Junak et al. 1995, Morrison 2007). Argentine ants likely add further stress to this ecosystem. For this reason, TNC and NPS have made eradication of Argentine ants a management priority.

Experimental Treatment and Control Monitoring Grids

We delineated a 4-ha treatment site at the Field Station, mirrored by an equivalent, untreated control area (Fig. 2). The treatment area was adjacent to the infested but untreated control area on a single side (~150 m in length), and the remaining sides of the treatment area contained low density or sparse Argentine ant nests. We conducted monitoring at 63 stations on a 20 × 40-m grid in the treatment area

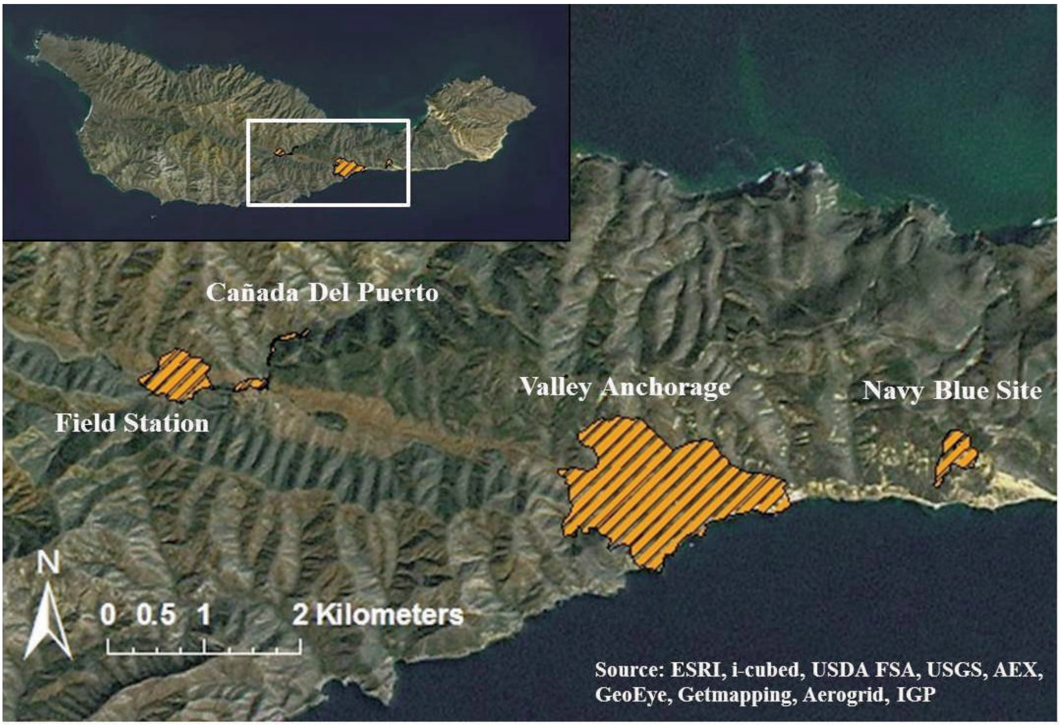

Fig. 1. Argentine ant infestations delimited on Santa Cruz Island, California, 2013.

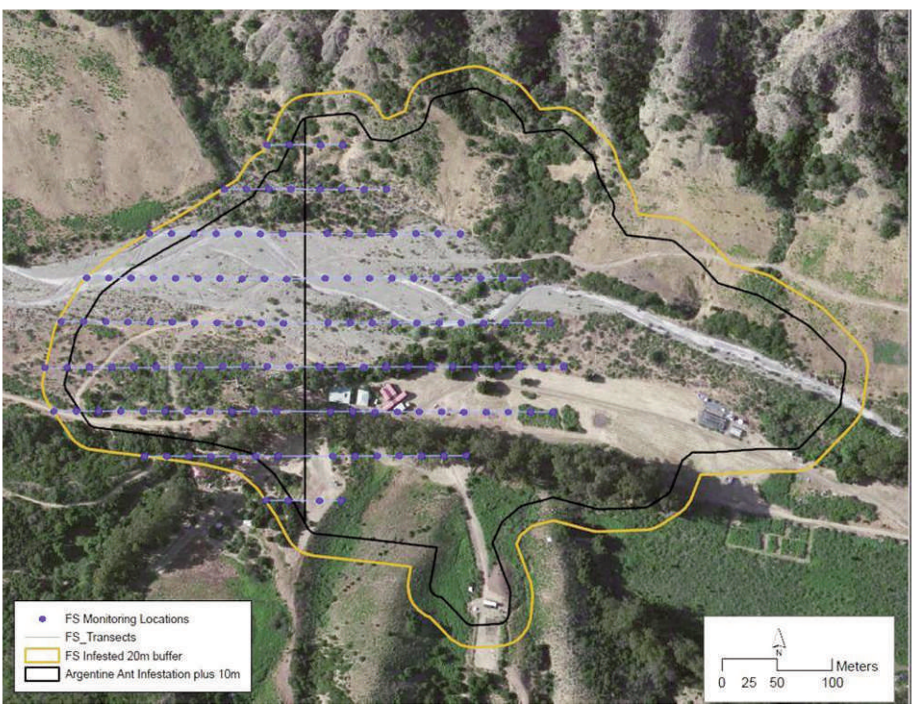

Fig. 2. Field Station infestation treatment and monitoring design on Santa Cruz Island, California.

and at 63 similar stations in the control site. The monitoring grid extended 20 m outside the edge of the treatment plot into uninvaded habitat, to account for errors in the delineation of the infestation boundary.

At the Navy Blue site infestation, we treated the north end of the infested area (approximately 3.8 ha). We established a 20 × 20-m monitoring grid which buffered the treatment area by 20 m, resulting in 131 monitoring stations. An untreated control plot was established within the Valley Anchorage infestation, about 3.5 km from the treatment area. An additional 131 monitoring stations were established in the untreated control site with a configuration like that of the Navy Blue site treatment plot.

Toxicant Bait Design

We deployed 2 toxicants in a 25% sucrose attractant. One toxicant was Optigard Liquid Flex (Syngenta Crop Protection LLC, EPA Reg. No. 100-1306) packaged at a concentration of 21.6% thiamethoxam and diluted to 6 parts per million (ppm) thiamethoxam with the 25% sucrose solution. We deployed this bait at a rate of 15 L per acre (0.4 ha) in polyacrylamide Water Storing Crystals (Miracle-Gro®). The small (~0.5-cm) polyacrylamide crystals absorb water and water-soluble chemicals. When hydrated, the polyacrylamide presents a thin layer of liquid bait solution on its surface for several hours until eventually drying out. They are designed as a soil amendment and can remain in the soil without any anticipated negative effect to native ecosystems. We also deployed thiamethoxam at 6 ppm in the 25% sucrose solution in polyvinyl chloride (PVC) bait stations. The PVC bait stations were made of 41-cm lengths of 3.8-cm diameter PVC pipe capped at both ends with 4 small holes drilled into one end and a small strip of spongy material in the tube cap to make the bait more available to ants. We deployed thiamethoxam on TNC land under a Research Authorization from the California Department of Pesticide Regulation No. 1204015 and on TNC and NPS lands under a letter of exemption from the U.S. EPA. We also used insect growth regulator S-Methoprene in the PVC bait stations according to label specifications in 2 separate baiting events in May and October. We diluted the product Tango (Central Life Sciences; EPA Reg. No. 2724–420) to 0.025% S-Methoprene in a 25% sucrose solution.

Bait Application

We deployed PVC bait stations at the Field Station site (n = 62) and the Navy Blue site (n = 20). We placed the PVC bait stations at 15-m intervals within areas of woody vegetation and left them in the field for approximately 30 days. Each station was cleaned, refilled, and relocated after each treatment to maximize bait coverage in the treatment area over the course of the summer. We filled the PVC bait stations with S-Methoprene for 2 treatments (May and October), and with thiamethoxam for 4 treatments (June–September).

We deployed thiamethoxam in polyacrylamide monthly from June through September for a total of 4 treatments. We carried the polyacrylamide in 5-gallon buckets and placed the bait in 15-mL piles using long-handled scoops at 2-m intervals in a grid pattern throughout the treatment areas. The piles were designed to reduce dehydration of the polyacrylamide and to give the ants more time to feed.

L. humile Activity Indices

We used 2 methods to assess ant activity within each site: nontoxic monitoring baits and pitfall traps. We assumed these ant activity measures were indices for ant abundance. We conducted monitoring approximately every 4 weeks May–October 2012 (for 6 total monitoring periods) immediately prior to treatment and again in May 2013.

NONTOXIC MONITORING BAITS.—In 6 monitoring rounds occurring approximately every 4 weeks (May 2012–October 2012), we placed 3 types of nontoxic attractants at monitoring stations (as described above) approximately 24–48 h prior to each treatment. We conducted monitoring during the early morning and late afternoon when temperatures were usually 18–26 °C. The attractants included (1) cotton balls soaked in 25% sucrose (carbohydrate), (2) natural peanut butter (protein), and (3) Xstinguish gel bait matrix without toxicant (Bait Technology, NZ) (carbohydrate and protein). We used peanut butter as protein bait rather than the more attractive tuna fish due to the large numbers of *Vespula* spp. that were observed to quickly consume tuna. We placed attractants in labeled 50-mL tubes. We randomly assigned each bait type to a fixed point 1 m apart in a triangle centered on the grid point. Approximately 1.5 h after deployment, we collected and capped the tubes and estimated the number of *L. humile*. In May 2013, we placed cotton balls soaked in 25% sucrose at all monitoring stations but did not deploy peanut butter or Xstinguish.

PITFALL TRAPS.—We placed pitfall traps at alternate monitoring stations (n = 31 at the Field Station and n = 76 at the Navy Blue site). We filled a 50-mL centrifuge tube with a soapy water solution, deployed a tube at every other monitoring station for 48 h during all monitoring periods (May–October 2012, May 2013), collected the tubes after 48 h, and counted and identified to species the ants in each tube.

Bait Attraction and Nontarget Species Monitoring

To determine how long *L. humile* fed on the polyacrylamide piles and which nontarget species might also consume the bait, we completed instantaneous counts of ants within 3 cm of each polyacrylamide pile placed at alternative monitoring stations (n = 31 at the Field Station, n = 76 at the Navy Blue site). We performed these counts at 2, 4, 6, 8, 24, and 48 h after deploying polyacrylamide piles. We identified to order and counted all insects at the bait.

Statistical Analyses

We analyzed variation in mean pre- and posttreatment ant activity measures for both the monitoring bait and pitfall indices with a blocked repeated-measures general linear model (GLM), using *L. humile* treatment as the fixed factor (treatment versus control), site as the blocking factor (Field Station and Navy Blue site), and time as the repeated-measures factor. To examine the variation in mean ant activity at each individual monitoring round, we performed separate Mann–Whitney U tests. To buffer for possible effects of reinvasion of treated areas from adjacent untreated areas, we performed identical analyses excluding measures taken within 20 m of the edges of the treated areas.

We examined changes in Argentine ant visitation to monitoring stations over the course of the efficacy trials by performing a one-way ANOVA with the cumulative Argentine ant instantaneous count data per monitoring point as the dependent factor and month as the fixed factor. To examine changes in Argentine ant visitation within the first 48 h of bait deployment, we performed separate one-way ANOVAs using the percent of Argentine ant visits as the dependent variable and hour after bait deployment (2, 4, 6, 8, 12, 24) as the fixed factor for each monitoring month.

We calculated the change in the mean Argentine ant activity after treatment at a grid point relative to before treatment as follows:

$$\% \text{ Pretreatment} = [(\text{mean activity posttreatment})/(\text{mean activity pretreatment})] \times 100.$$

We calculated the difference of the Argentine ant activity between the paired control and treatment plots over time as follows:

$$\text{Abundance Ratio } \% = [(\% \text{ Pretreatment in treatment plot})/(\% \text{ Pretreatment in control plot}) - 1] \times 100.$$

When abundance ratio % = 0, there is no difference between control and treatment plots.

RESULTS

The protocol yielded an immediate and sustained reduction in the Argentine ant activity within treatment plots as measured by ant numbers at monitoring baits and in pitfall traps. Changes in Argentine ant activity within monitoring baits (Argentine ant treatment × time GLM: $F_{5,1360} = 38.05$, $P < 0.001$) and pitfall traps (Argentine ant treatment × time GLM: $F_{5,655} = 21.91$, $P < 0.001$) differed significantly among treatments through time (May–October 2012) and among each discrete monitoring period following the initial thiamethoxam bait deployment in June 2012 (Mann–Whitney U test: $P < 0.001$ in all cases; Fig. 3). Approximately one year after we initiated the protocol, the abundance of Argentine ants was reduced in the treatment plots compared to untreated plots by 99.996% (SE 0.004%) within 25% sucrose monitoring baits and 99.896% (SE 0.052%) within pitfall traps (Fig. 3). We recorded 59 individual Argentine ants at monitoring stations (25% sucrose and pitfall traps) in the treatment plots in May 2013. When we excluded monitoring stations that could have experienced reinfestation from untreated edges (defined as a 20-m interior buffer around the perimeter of the treated

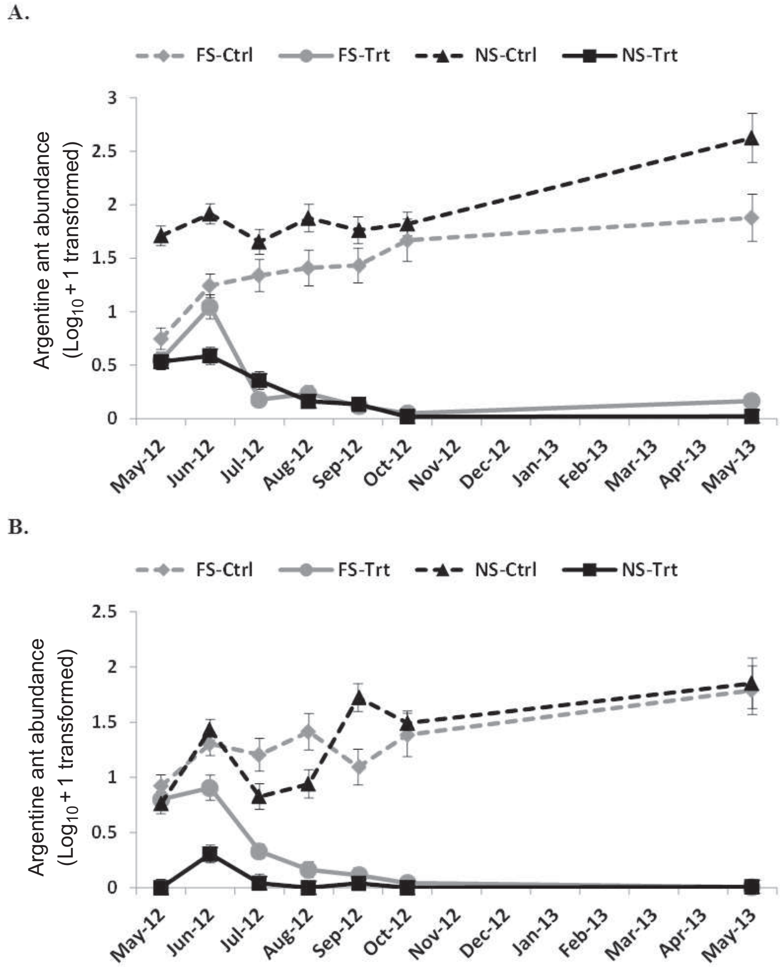

Fig. 3. Mean (±1 SE) Argentine ant abundance ($\log_{10}$+1 transformed) within the 2 control (Ctrl) and treatment (Trt) plots collected from (A) pitfall traps and (B) monitoring baits at the Field Station (FS) and Navy Blue site (NS), Santa Cruz Island, California.

areas), we collected 1 ant at the treated Field Station site and 4 ants in the treated Navy Blue site, whereas approximately 216,700 Argentine ants were collected in the untreated sites. An average increase of 1301% (SE 716%) in the number of ants collected with 25% sucrose monitoring baits and 16,980% (SE 6673%) with pitfall traps in the untreated plots in May 2013 compared to the same time in 2012 may be the result of ants being attracted to the monitoring stations due to extended drought.

We observed significantly different numbers of Argentine ants visiting the polyacrylamide piles between months ($F_{3,575}$ = 56.963, P < 0.001) and between hours after bait deployment within a given treatment ($F_{5,505}$ = 41.614, P < 0.001). More Argentine ants visited the polyacrylamide piles in June during the first thiamethoxam treatment than in all other monitoring months (P < 0.014 in all cases; Fig. 4). The number of Argentine ants visiting the polyacrylamide piles was higher within 2 and 4 h of bait deployment than in all other post–bait deployment monitoring rounds (P > 0.001 in all cases; Fig. 5). In total, we observed 66% (SE 5%) of all Argentine ant visitations within 4 h of bait deployment. Nonetheless, Argentine ants did visit the toxicant bait 24 and 48 h post–bait deployment.

We only observed arthropods visiting the polyacrylamide piles during nontarget monitoring. In total, 94.1% of the individuals we

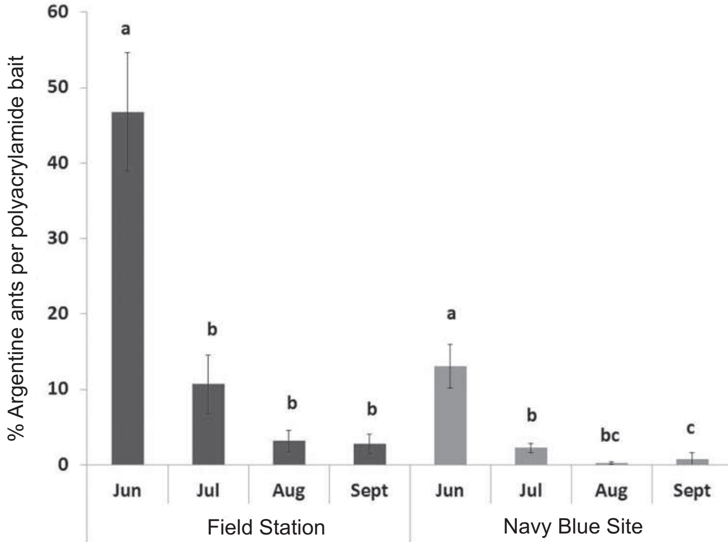

Fig. 4. Mean (±1 SE) Argentine ant abundance per instantaneous bait count on polyacrylamide piles at the Field Station and Navy Blue site, Santa Cruz Island, California. Bars representing each site (Field Station and Navy Blue site) with different letters are significantly different (post hoc Tukey tests: $P < 0.05$).

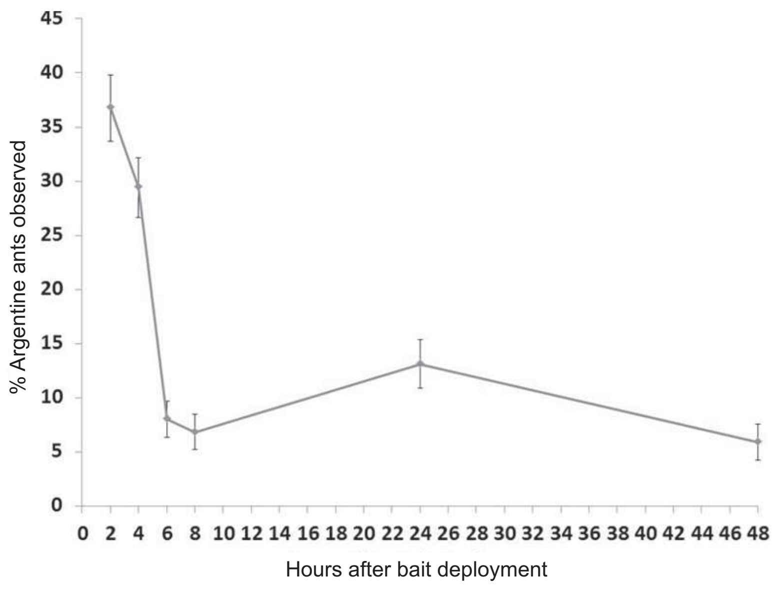

Fig. 5. Mean (±1 SE) percentage of the instantaneous count visitation over the first 48 h of toxic bait deployment at the Field Station and Navy Blue site, Santa Cruz Island, California.

recorded during the 3267 instantaneous toxicant bait counts in June–September were ants. The remaining 5.9% consisted mainly of isopods and other abundant and nonsensitive arthropods. The percentages of the total visitors varied among taxa ($F_{12,91} = 18.206$, $P < 0.01$), and *L. humile* was the most frequent visitor (Fisher's LSD: $P < 0.01$ in all cases). When Argentine ants were present on the toxicant bait, significantly fewer nontarget species visited the bait compared with when Argentine ants were absent (Wilcoxon's signed rank test: $Z =$

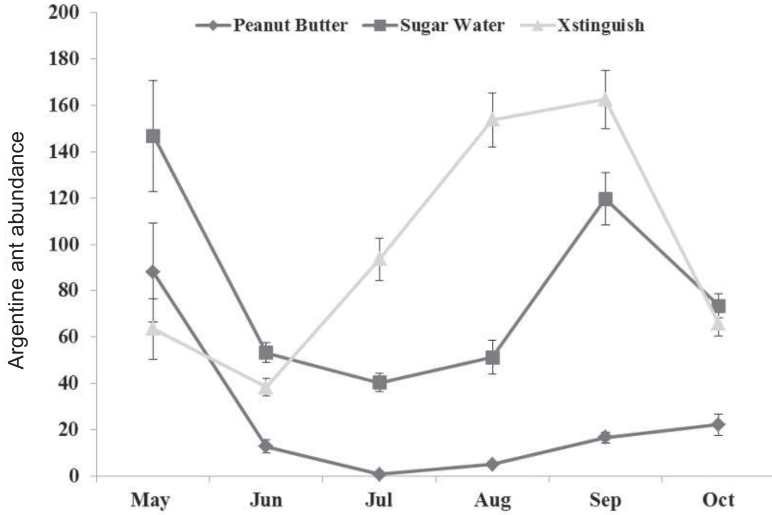

Fig. 6. Mean (±1 SE) Argentine ant abundance for the 3 monitoring baits (i.e., peanut butter, sugar, Xstinguish) within each monitoring month at the UC Field Station and Navy Blue site, Santa Cruz Island, California.

2.12, $P = 0.012$). Consequently, we hypothesized that the visitation rates of nontarget species would increase with subsequent treatments as Argentine ants visitation rates decreased. However, visitation rates from nontarget species also decreased with each toxic bait deployment (taxa × time ANOVA: $F_{3,8} = 1.185$, $P = 0.29$).

The most abundant nontarget group observed visiting the polyacrylamide piles were native ants, and these were more abundant on baits in areas where Argentine ants were absent. Native ants were significantly more abundant in pitfall traps on the edges of the treated areas compared with the interior sections (Mann–Whitney *U* test: $U = 626.5$, $P = 0.005$). In total, 82.0% (SE 3.6%) of the native ants collected in pitfall traps occurred along the edges of treated areas where Argentine ants were largely absent. When we excluded the data from monitoring stations where *L. humile* were never observed (mostly stations outside of the treated areas), Argentine ants represented 79.0% and 3 native ant species represented 18.9% of all visitors (Fig. 6b).

Numbers of Argentine ants collected in monitoring tubes May 2012–October 2012 differed significantly among nontoxic bait types (i.e., sucrose, peanut butter, Xstinguish) through time (monitoring baits × time GLM: $F_{10,1000} = 10.968$, $P < 0.001$; Fig. 6). The attraction of the monitoring baits differed significantly during every monitoring round (two-way ANOVA: $P < 0.002$ in all cases). Sucrose and Xstinguish were significantly more attractive than peanut butter during all monitoring rounds (paired *t* test: $P < 0.02$ in all cases). Sucrose was significantly more attractive than Xstinguish bait in May ($t_{124} = 2.767$, $P = 0.007$) and June ($t_{259} = 3.097$, $P = 0.007$); but Xstinguish bait was significantly more attractive than sucrose in July ($t_{213} = -5.888$, $P < 0.001$), August ($t_{193} = -9.807$, $P < 0.001$), and September ($t_{188} = -2.743$, $P = 0.02$). There was no significant difference between the numbers of ants attracted to sucrose and Xstinguish monitoring baits in October 2012 ($t_{182} = 1.492$, $P = 0.412$).

DISCUSSION

Our protocol—a combination of the baited polyacrylamide, PVC-pipe bait stations, 6-ppm thiamethoxam, 0.025% S-methoprene, and the attractant 25% sucrose solution—reduced *L. humile* activity in both high- and low-abundance infestations. The initial reduction in ant activity observed in the treated areas after the first treatment (78%) was similar to reported results using other bait and attractants (Krushelnycky et al. 2011). Subsequent bait deployments further reduced ant activity, indicating that the treatment affected smaller nests with fewer workers. Searches of randomly selected areas within the treatment plots in October 2012 and May 2013 revealed Argentine ants in only one area in the Field Station site, confirming the results observed in bait station and pitfall trap monitoring. We recorded similar reductions in ant activity measures (monitoring stations and pitfall traps) at the Field Station and the Navy Blue sites over the course of the treatments, despite differences in vegetation and initial ant activity results. These data present a strong case for the utility of this protocol as an eradication tool, indicating that it can both reduce ant activity in highly infested areas and affect small or dispersed nests in coastal sage scrub and scrub oak–chaparral habitats.

Posttreatment field observations indicated that ants continued to feed on the 25% sucrose monitoring baits throughout the treatment trial (June–October) with no evidence of a "bait shyness" response, described by pest control professionals as an aversion to the bait for several weeks or months following treatment (V. Van Dyke, Bait Technology Ltd, personal communication). Although we observed fewer ants on polyacrylamide piles in later monitoring periods, the decline in recorded observations was consistent with the multiple activity indices, suggesting this may have been a result of reduced ant numbers. If ants display consistent bait uptake, frequent bait deployments may be used to increase the likelihood that the bait will be consumed by every queen.

In addition to systematically sampling the treated and control areas for ant activity using monitoring baits and pitfall traps, we manually searched for ants during all monitoring periods. Dry, sparsely vegetated areas were the first areas where we recorded no *L. humile* during visual inspection. In the October monitoring round, foraging trails were not observed in any area of either treatment site except one area on the north side of the Field Station treatment site populated by 4 coast live oak trees with canopies that encompass approximately 250 m^2. An extensive 3-dimensional habitat, locally abundant resources, or large pretreatment nests may have rendered our treatment less effective in that area. We could more effectively reduce ant activity in that area by (1) placing bait in that 3-dimensional habitat in quantities and coverage mimicking the 2 × 2-m grid we placed on the ground of the treatment areas or (2) increasing the attractiveness of the bait by adding protein.

Initial trials of an attractant consisting of a mixture of 50% cooked and 50% raw chicken egg and 25% sugar by weight indicate this is a highly preferred food source (Hanna and Boser 2012), and our monitoring data indicate that protein and sugar attractant is preferred in July–September. The consistency of the cooked and raw sugared egg solution makes the attractant easily spread or sprayed, and the solution tends to adhere to vegetation which makes it suitable for deploying on 3-dimensional surfaces. Although more costly than the sucrose bait, this bait could be considered for use during July–September to target remnant nests in inaccessible locations or those not effectively eliminated by the sucrose bait.

Our field observations indicated that Argentine ants visited the PVC bait stations less than the polyacrylamide bait piles. Although there were fewer PVC bait stations placed farther apart than polyacrylamide bait piles, the bait smelled of vinegar after a few days; so the large volume of bait placed in the PVC bait stations was not fully utilized by the ants. When ant abundance is low or nests are small, ants may not forage more than a couple meters from their nests (C. Green personal communication), so it is likely many nests could not access a PVC bait station. Considering the limited visitation and the long handling time needed to deploy the PVC bait stations, this deployment method was not cost-effective.

The effect on nontarget organisms was small, and most of the expected invertebrate mortality is assumed to be compensatory. The majority of nontarget ant species were recorded only on the periphery of the treatment sites where Argentine ants were absent or observed at low densities. Typically only 1–3 ant species coexist with Argentine ants in infested locations on Santa Cruz Island (Holway and Hanna 2011). We hypothesize that very few native ant species will persist in areas in which Argentine ants spread. Thus we consider most native ant mortality to be compensatory rather than additive. With the exception of a single observation of an unidentified beetle (Coleoptera) on bait during instantaneous bait counts, all of the nontarget invertebrates observed on the bait are highly abundant and widespread across Santa Cruz Island. We do not expect significant changes in invertebrate assemblages as a result of the treatment other than elimination of Argentine ants and the resulting positive impacts to native species.

Our pretreatment review of the literature and posttreatment observations indicated that the baiting treatment would not have significant or detectable effects on vertebrate species. Oral LD50 levels measured for the active ingredient thiamethoxam in birds and mammals is 576 and 5000 mg · kg^{-1} body weight, respectively. We estimate an average common raven (*Corvus corax;* at 1 kg) would be required to consume 12.9 L and an island fox (*Urocyon littoralis;* at 2 kg) 1374 L of 6-ppm bait to reach LD50 levels. We recorded one observation of a raven consuming the bait but at a much lower quantity than would be required to meet LD50 levels. No other bird was observed to consume the bait, even though several observation sessions were conducted in areas where ground-foraging birds typically feed.

The island fox is on the federal and state endangered species list, and for this reason we conducted a number of tests to assess the effects of the polyacrylamide on the foxes prior to the large-scale baiting. During 30 trapping nights, remotely triggered cameras were placed facing a 30 × 30-m area baited with polyacrylamide and 25% sucrose solution. Foxes were recorded consuming the polyacrylamide on 10% of trapping nights for an average of 4.5 minutes each night. Additionally, we used standard trapping procedures (Coonan et al. 2005) for 4 nights following the first polyacrylamide bait deployment in June to monitor the effects of the treatment on foxes. Trapping was conducted 40 nights in the Field Station treatment area. None of the 11 individuals we trapped showed intestinal distress, and fox capture rates were normal. To date there is no evidence of adverse effects on island fox; however, we continue to monitor fox health in the vicinity of the bait deployments.

In our field trial, as in programs carried out elsewhere, reinfestation from the untreated infestation edges appeared to confound monitoring results (Krushelnycky et al. 2011). To better measure the true efficacy of a treatment, the treatment buffer could be extended around the monitoring stations, but that treatment buffer must be modified and increased relative to the duration between treatment and final monitoring to account for reinfestation rates of 10–100 m per year. In many areas, the size and shape of the infestation makes this difficult, so it is preferable to treat the entire infestation to ascertain treatment efficacy. In these field trials, all but 5 of the Argentine ants recorded in the final May 2013 monitoring round were recorded on the periphery of the infestation. We recommend that further trials be conducted on entire infestations to reduce uncertainty about reinfestation influencing monitoring results.

Defining the treatment boundary and adding a suitable buffer to the treatment area remains an important and challenging component of Argentine ant treatment. The treatment boundaries we established prior to this trial were based on delimitation data which was conducted in October 2010, nearly 18 months prior to our initial treatment. Although we did not complete a delimitation immediately prior to the experiment, we recommend mapping Argentine ant infestations immediately prior to treatment to improve the ability to discern efficacy. Field observations indicate that Argentine ant nests on Santa Cruz Island are not necessarily adjacent to each another. The Argentine ant invasion front may be undetectable for up to 30 m in less suitable habitat such as annual grasses, but small colonies at the leading edge of the infestation may still exist at more distant locations around native shrubs or trees. Some pest professionals recommend a 20-m treatment buffer around delineated Argentine ant populations. Based on our field observations and the results of ant activity monitoring stations, we recommend using a larger treatment buffer of 50 m.

Management Implications

The history of ant eradication attempts elsewhere underscores the importance of long-term commitment to treatment and monitoring. Our results from this treatment trial—particularly the consistency of the results across the 2 treatment sites and different vegetation types, as reflected with different monitoring methods—suggest that we may have a practical and effective method that could make elimination feasible. At the very least, the results support the continuation of this experiment on a larger scale. Indeed, we expect that several additional treatments with this protocol will be necessary to entirely eliminate all evidence of colonies at the previously treated sites. We will also examine the return on investment of increasing the frequency of treatments within a given season. Our results suggest that this protocol, or variations thereon, may be effective in eliminating colonies of Argentine ants. The polyacrylamide bait piles appear to be a practical and effective method of deploying attractive liquid bait in rugged terrain and dense vegetation. If Argentine ants can be eliminated on the scale of our field trials, we expect the protocol could be scalable to larger isolated infestations on islands and other conservation areas. Moreover, we expect that the polyacrylamide bait deployment method could be adapted to control or eliminate populations of other invasive ants.

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