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The Haleakala Argentine ant project: a synthesis of past research and prospects for the future

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EXECUTIVE SUMMARY

1. The Haleakala Argentine Ant Project is an ongoing effort to study the ecology of the invasive Argentine ant in the park, and if possible to develop a strategy to control this destructive species.

2. Past research has demonstrated that the Argentine ant causes very significant impacts on native arthropods where it invades, threatening a large portion of the park's biodiversity in subalpine shrubland and alpine aeolian ecosystems.

3. Patterns of spread over the past 30+ years indicate that the invasion process is influenced to a substantial degree by abiotic factors such as elevation, rainfall and temperature, and that the ant has not reached its potential range. Predictions of total range in the park suggest that it has only invaded a small fraction of available suitable habitat, confirming that this species is one of most serious threats to the park's natural resources.

4. Numerous experiments have been conducted since 1994 in an attempt to develop a method for eradicating the Argentine ant at Haleakala using pesticidal ant baits. Thirty baits have been screened for attractiveness to ants in the park, and ten of these were tested for effectiveness of control in field plots. While some of these baits have been very effective in reducing numbers of ants, none has been able to eliminate all nests in experimental plots.

5. Research into a secondary management goal of ant population containment was initiated in 1996. By treating only expanding margins of the park's two ant populations with an ant pesticide, rates of outward spread were substantially reduced in some areas. While this strategy was implemented from 1997 to 2004, it was ultimately discontinued after 2004 because of the difficulty and insufficient effectiveness of the technique.

6. In order to achieve the types of results necessary for eradication, the project would probably need to explore the possibility of developing a specialized bait, rather than relying on a commercially produced bait. An alternative would be to pursue approval to use Xstinguish bait, a commercial bait manufactured in New Zealand and not registered for use in the US, which has yielded good results against Argentine ants. Either route would involve significant regulatory hurdles. Because the baits ultimately used would likely be liquid or paste in form, there would also be major logistical challenges in devising methods to successfully apply the baits across the two large ant populations at Haleakala.

I. INTRODUCTION

The Haleakala Argentine Ant Project has been an effort, spanning the past 30 years, to understand the ecology of the invasive Argentine ant in Haleakala National Park (HALE) and, if possible, to develop management solutions for this important threat to park natural resources. Although the Argentine ant was recognized as a serious cause for concern soon after its detection in the park in 1967, research into management did not begin in earnest until the mid 1990's. Some of this research on Argentine ant control at HALE has previously been published in journal articles or agency reports, but much of it is reported for the first time here. This technical report provides an overview and summary of all of the Argentine ant control efforts that have taken place at HALE from 1994 through 2010.

II. BACKGROUND

It is believed that there are no native ants in Hawaii. Nearly 60 ant species have been introduced to the state in the past several hundred years, however, and some of these have caused substantial impacts to native Hawaiian biodiversity (Reimer 1994, Krushelnycky et al. 2005a). Most of the ant species introduced to Hawaii have thrived in the warmer lowlands, and have probably contributed to the extinction of numerous native insects and related arthropods. A handful of species are better adapted to cooler climatic regimes, and several of these become extremely abundant, dominant invaders in Hawaii's middle to high elevation zones. The Argentine ant, *Linepithema humile* (Mayr), is among the latter group, and has become one of the most important threats to subalpine shrubland and alpine zone ecosystems in Hawaii.

The Argentine ant was first recorded in the park at Hosmer Grove in 1967 (Huddleston and Fluker 1968). Because this ant had such a notorious reputation as a destructive invasive species in California, the southeastern U.S., Australia, Europe, and South Africa, HALE staff and collaborating scientists initiated several research projects in order to learn more about its invasiveness and probable impact in the park. Beginning in 1980, researchers periodically mapped the ant's distribution to track its spread. Subsequently, studies were conducted in the mid 1980's and again in the mid-2000's to estimate the ant's effects on the resident arthropod community. The results of these efforts confirmed that the Argentine ant was indeed one of the most important threats to the park's endemic biota; a wide range of native arthropods were significantly reduced in abundance as the ant continued to steadily spread into larger areas of the park, with wider ecological impacts possible. These results are summarized below.

A. Patterns of spread

The record of Argentine ant invasion at HALE represents one of the most detailed accounts of alien species invasion. The two ant populations have been mapped periodically from 1980 to 2004, with increasing frequency and accuracy since 1993. Synthesis of these data highlights several important trends. First, the patterns of spread clearly indicate that this species is not restricted to areas of human disturbance, as is sometimes the case with invasive ants. The vast majority of the two populations occupy habitat unaffected by roads, trails or structures. Second, abiotic factors, most importantly rainfall and soil temperature, have strong influences on where, and at what rate, the ant populations spread within the park.

When the Argentine ant was first mapped in 1980, thirteen years after its detection at Hosmer Grove, it occurred over an area of about 165 ha that encompassed the park service area and headquarters (Fellers and Fellers 1982). The Argentine ant, like several other invasive ant species, does not have mating flights, and its populations therefore spread through budding: newly mated queens disperse with a retinue of worker ants a short distance on foot, creating a general pattern of outward population expansion. In 1982, a second ant population was discovered higher up the mountain, perched on the crater rim around Kalahaku Overlook (Fellers and Fellers 1982). This second population was recent in origin and still fairly limited in distribution, and was most likely initiated through inadvertent human movement of nest material from the first population. Over the fifteen years from 1982 to 1997, both populations increased dramatically in size, reaching a total area of about 555 ha. This spread is illustrated in Figures 1-3 in Appendix 1. (We have designated the two ant populations as the lower population and upper population, referring to the original and secondary populations, respectively).

The pattern of spread in the lower population indicates that the Argentine ant appears to have an upper rainfall tolerance of about 1600 mm/yr at this location. Hawaii’s northeast tradewinds create the predominant weather patterns on the mountain: the north and northeast slopes of Haleakala receive high levels of annual rainfall, while the leeward south and southwest slopes sit in a rain shadow and are considerably drier. In between these areas, such as at Hosmer Grove on Haleakala’s northwest slope, steep rainfall gradients are formed (Appendix 1, Fig. 1). Over the course of 30 years, the lower ant population has not spread to the northeast beyond Hosmer Grove. Instead, 94% of the eventual colonization occurred southwest of Hosmer Grove, or towards the lee of Haleakala volcano (Appendix 1, Fig. 2). Spread towards the northeast is most likely limited by cold soil temperatures, which result largely from the increased vegetative cover that prevails as rainfall increases along the windward gradient.

Unlike the lower population, the upper population has not yet encountered environmental conditions that prevent its outward expansion. However the *rate* of spread within the upper population has not been equal in all directions. Namely, expansion from Kalahaku overlook westward outside the crater rim has been relatively slow (averaging 23 meters per year), while eastward spread down the crater walls and across the crater floor has been much more rapid (increasing to 91 meters per year) (Appendix 1, Fig. 3). Rates of spread in both populations are strongly tied to soil temperatures, and can be predicted with fairly high accuracy using a simple degree-day model based on soil temperature. As the upper population has reached warmer temperatures down the crater walls and on the crater floor, rates of population growth and opportunity for above-ground foraging have likely increased, resulting in the faster rates of spread. Similarly, the lower population has expanded more rapidly towards the lee of the volcano, where sparser vegetative ground cover results in warmer soil temperatures.

Based on these past patterns of spread, we made a rough estimate in 2004 of the total potential range of the Argentine ant in the park (Appendix 1, Fig. 4), as well as the rate at which it would likely fill this range. As indicated in Figure 4, this potential range included most of the remainder of the west slope shrubland and aeolian zone, including the summit, and much of the crater and

Kaupo Gap. At 5360 ha, the predicted potential range encompassed approximately 48% of the total park area (based on the park land holdings at the time), and 75% of the park's subalpine shrubland and alpine aeolian zone habitats. More recent modeling of potential range based on additional soil temperature measurements and degree-day estimates (Hartley et al. 2010) suggests that this earlier estimate was probably conservative, and that even more of the park is suitable for Argentine ant invasion. Moreover, suitable habitat will increase as temperatures increase at the top of Haleakala, and this warming is currently occurring at a rapid rate (Giambelluca et al. 2008). We also estimated that without management intervention, the two populations would merge on the west slope in less than 20 years, would invade half of the predicted total range in the park in 30 years, and would cover roughly 90% of this area in about 70 years. We estimate that the establishment of the second population at Kalahaku around 1977 has accelerated the invasion process into the park by about 50 to 60 years, underscoring the critical importance of preventing additional founder populations. A more detailed account of these trends and predictions is provided in Krushelnycky et al. (2005c) and Hartley et al. (2010).

B. Ecological effects

In the mid-1980's park scientists and colleagues used pitfall sampling and under-rock surveys to assess the effects that the Argentine ant invasion was having on the arthropods in the shrubland and alpine aeolian ecosystems (Medeiros et al. 1986, Cole et al. 1992). They found that a wide range of endemic arthropods is significantly reduced in abundance within ant-invaded areas. The affected groups include, among others, herbivores (*Agrotis spp*. [Lepidoptera: Noctuidae]), predators and scavengers (*Mecyclothorax* and *Blackburnia spp*. [Coleoptera: Carabidae], *Lycosa hawaiiensis* Simon [Araneae: Lycosidae]), and pollinators (*Hylaeus spp*. [Hymenoptera: Colletidae]). These results were largely substantiated, and expanded upon, in studies conducted in the mid-2000's (Krushelnycky and Gillespie 2008, 2010). These latter investigations found that native arthropod species richness declines by over 50% in ant-invaded areas of the park. In addition, the overall abundance of native arthropods drops dramatically, and communities become numerically dominated by introduced arthropods. Affected native species represent all trophic groups, and include species active on the ground, inhabiting the leaf litter, and even those resident on shrubs. Rare endemic predators are most vulnerable, but up to 65% of native species overall are drastically reduced in abundance in invaded areas.

Many Hawaiian arthropod species have very limited distributions (Howarth 1990), and the expansive populations of Argentine ants could easily spread to occupy the entire natural ranges of some of HALE's most localized and rare endemic species, placing them at high risk of extinction. Recently, three species of native carabid beetles have been rediscovered adjacent to the Argentine ant populations at HALE after going undetected for over 100 years (Krushelnycky et al. 2005b). None of these rediscovered carabid species have been found within ant-invaded areas, and most appear to have extremely localized distributions. If the ongoing spread of the two ant populations cannot be halted or reversed, we may be placed in the unfortunate position of watching these beetle species go extinct. Moreover, the invasion by Argentine ants of the majority of the subalpine shrubland and aeolian zone habitats at the top of Haleakala, as predicted, has the potential to eliminate other spectacular examples of Hawaiian evolution. For instance, a flightless moth that hops around the cinders (*Thyrocopa apatela* [Walsingham]), a flightless lacewing with spiked, beetle-like forewings (*Micromus cookeorum* [Zimmerman]), and a silversword-feeding long-horned beetle (*Plagithmysus terryi* [Perkins]) are only found there.

Argentine ant invasion has also led to community-level impacts at HALE. Arthropod trophic structure is strongly altered in invaded areas, as is overall arthropod biomass (Krushelnycky and Gillespie 2008). These alterations signal changes in energy flow and, potentially, changes in ecosystem function in invaded areas. One hypothesized functional impact is that Argentine ants may negatively affect reproduction of obligate outcrossing plant species, such as silverswords, by reducing numbers of native pollinators (Cole et al. 1992). For instance, seemingly moderate reductions in rates of silversword seed set (e.g. from 30% to 20%) are predicted to result in dramatic population declines over the long term (Forsyth 2002). More recent work indicates that Argentine ants are not currently depressing silversword seed set rates (P. Krushelnycky unpub. data). However, ants have only invaded a small portion of silversword habitat so far, and future expansion into much larger areas of silversword habitat could very well have a much stronger impact on the silversword's pollinators and reproduction.

In the early 1990's park staff became concerned about the spread of the upper ant population into endangered Uau (or Hawaiian petrel, *Pterodroma sandwichensis*) nesting habitat. Invasive ants have been found to prey upon the nestlings of a number of ground-nesting birds (Holway et al. 2002), and the Argentine ant could therefore pose a significant new threat to this imperiled bird that constructs burrows in Haleakala's cliffs. Monitoring of nesting colonies both within and outside the ant population by USGS and NPS, however, revealed that the Uau currently appears to be unaffected by ants, with similar reproductive success rates in both areas (Krushelnycky et al. 2001). In this case, cold temperatures within the petrel burrows likely discourage heavy ant foraging to the nest chambers. This situation may need to be re-examined as temperatures continue to increase on upper Haleakala, because petrel burrows may begin to become thermally accessible to ants.

III. RESEARCH ON ARGENTINE ANT MANAGEMENT AT HALE

A. Rationale for attempting ant control

As the implications of the Argentine ant invasion became clear, research focus shifted towards developing a technique to control, and ideally eradicate, the ant in the park. Because of their budding modes of dispersal, populations of some invasive ant species like the Argentine ant can occur as discrete entities even when their overall distributions are wider. The lack of mating flights means that eradication of incipient populations could result in the permanent removal of these species from particular areas of concern, as long as re-introduction by humans can be prevented or quickly detected. In the 1980's and 1990's, for example, a 3 ha population of the invasive little fire ant (*Wasmannia auropunctata*) was eradicated from the Galapagos island of Santa Fe using an ant pesticide (Abedrabbo 1994). This success was an important impetus for the Argentine ant project at HALE. The two populations in the park are well isolated from other Argentine ant invaded sites on the island, and a similar technique could possibly result in substantial control, or even eradication, of Argentine ants at HALE. Numerous additional ant eradication campaigns, many with explicit conservation goals, have been conducted since the early success on Santa Fe Island (Hoffmann et al. 2010). Most of these, however, have been much smaller in scale compared to the Argentine ant infestation at HALE.

The use of pesticides may seem to contradict the conservation mandate of a national park. However, the control of invasive species often represents the most critical management actions required in Hawaii's protected areas. Moreover, pesticides developed for ant control have several important improvements over previous wide-spectrum contact insecticides. These pesticides are developed as baits that are attractive to ants, and are typically formulated with only a small amount of active ingredient (toxicant). Ants locate the baits while foraging and bring them back to the nest to share among the colony, meaning that relatively small amounts of the baits need to be used to reach all of the target nests. And because the baits are often highly attractive to the ants and invasive ant populations often achieve very high densities, the target ants are much more effective at retrieving and consuming the baits than non-target arthropods. These attributes have the effect of making ant baits both more effective and less likely to result in severe non-target impacts; ant baits have represented by far the most commonly used technique when attempting ant eradication in natural areas (Hoffmann et al. 2010).

Despite the improved safety of today's ant baits, it is virtually impossible to avoid all non-target impacts when using pesticides. Even if the target ant species retrieves all of the bait applied, secondary poisoning may occur among organisms that consume dead or moribund ants. In addition, if multiple applications of the baits are needed, the likelihood that some non-target arthropods will directly ingest the baits increases greatly. At HALE, these potential non-target impacts have been deemed to be tolerable because the ecological impacts from Argentine ants are so high. The same species that may be impacted by ant control efforts are likely already severely impacted by the ants themselves. Moreover, as summarized above, Argentine ants currently only occupy a small fraction of the total area that they will almost certainly eventually invade in the park. Some temporary non-target impacts from pesticide use is preferable to the long term impacts resulting from unrestricted Argentine ant invasion into the park.

B. General strategy

Successful control of invasive ants involves the seemingly simple objective of delivering an effective bait to the infested area. There are therefore two components that are inter-related and impinge on each other: finding or developing an effective bait, and devising an application system that can deliver the bait to the target area. The difficulties in actually achieving control or eradication lie not only in finding successful solutions to these two challenges, but also stem from the reality that the best solutions to the two challenges can be in conflict. The situation at HALE exemplifies this issue. Argentine ants are highly attracted to liquid foods, especially plant nectar and the sugary exudates of plant sap-sucking insects (Hemipteran honeydew). Not coincidentally, much Argentine ant control research has focused on developing sugar water-based baits (formulated with a toxicant), because this food source is usually avidly consumed by workers, is easily carried back to the nest internally in the ant's crop, and is readily shared with nestmates through trophallaxis (regurgitation). Liquid and gel/paste baits are therefore often more effective than drier granular baits against Argentine ants, simply because the baits are more attractive to the ants. However, liquid baits need to be delivered within bait stations, and gel or paste baits usually need to be delivered by hand. The size, topographic complexity and complete inaccessibility of parts of the two Argentine ant populations at HALE raise serious questions about whether any liquid, gel or paste baits could realistically be applied to the entire infested area. In contrast, aerial broadcast systems exist for granular baits, and granular baits could in theory be delivered to all infested areas at HALE. More effort has therefore been directed at testing granular baits at HALE, even though granular baits are expected to usually be less attractive and hence less effective than liquid or gel baits.

An effective ant bait must consist of a highly attractive matrix (consisting mostly of a food attractant) combined with a toxicant that is undetectable to foraging ants and which has delayed action, allowing foragers to bring the bait back to the nest and share it with nestmates, including queens and brood (immature stages), before they are killed. The ease of meeting these criteria varies among invasive ant species: some species are much easier to control and indeed eradicate than others. Unfortunately, Argentine ants have been found to be among the hardest of invasive ant species to control, with few cases of successful eradication. The reasons for this greater difficulty are unclear but could arise from a number of factors, possibly including a greater number of queens in each nest (some of which may receive food in reduced quantities or quality), a greater sensitivity and ability to respond to incipient mortality in the nest, and higher selectivity or variability in food gathering and consumption.

The challenge at HALE has therefore been to find a bait that is effective enough in experimental test plots to justify attempting eradication on a larger scale, and that at the same time could be applied to large areas in a feasible manner. This will usually mean that a prospective bait can kill all ant nests within test plots with one to several applications (if ants cannot be eradicated from relatively small plots, attempting eradication from hundreds of infested hectares in the park would be pointless), and that it could be applied aerially. A secondary, lesser goal, has been to find a bait that causes enough mortality to prevent outward spread of the two populations when applied to their perimeters. This containment strategy could greatly increase the chances of success of the first goal in the future, even if an eradication strategy is not available at present. Most of the management research summarized below has been directed at these two goals. From 1994 to the present, various baits have been assessed for effectiveness in experimental plots. These have met with varying degrees of success, but none has been able to completely eradicate all ant nests within the plots. Additional baits have been screened less extensively to assess basic attractiveness. Beginning in 1997, a moderately effective granular bait (Maxforce Granular Insect Bait) was broadcast once annually within a 120m wide perimeter zone in an attempt to slow the outward expansion of the two ant populations, while new baits continued to be tested for their potential to eradicate the populations. This containment strategy also met with varying degrees of success, and was discontinued after the summer of 2004 when results were especially poor.

As no easily broadcast (i.e. granular) baits appeared to be sufficiently effective, we began conducting research on liquid baits. The rationale for testing these baits was that it would be useful to know if liquid baits were more effective than granular baits at HALE, even if their application on a larger scale seemed unrealistic at present. In addition, effective liquid baits could be used to control ants in smaller sites that may be acting as high-risk source pools for initiating new populations in distant areas of the park. These include park buildings, greenhouses and campgrounds. The experimental bait trials and containment efforts mentioned above are elaborated in the following sections and in the attached appendices.

C. Testing baits for eradication potential

The tests summarized below all used similar monitoring methods, which are usually not detailed in the following sections to avoid redundancy. Unless otherwise noted, effects of the pesticidal ant baits were assessed with two methods: 1) monitoring of foraging ant numbers in the plots before and after application using index cards provisioned with a small amount of attractant bait, with no toxicant, and placed on the ground at regular intervals, usually in a grid pattern (referred to as 'bait card monitoring'), and 2) nest surveys, in which all rocks in a delineated portion of the plot were overturned in order to locate all nests and quantify abundances of all ant life stages observed (using abundance categories; life stages included workers, queens, males, eggs, larvae and pupae). For bait card monitoring, the number of monitoring stations per plot depended on the size of the plot, but were usually placed at 5 to 10 m intervals; the bait attractant placed on the cards was blended fermented fish from 1995 through 1998, and a blend of canned tuna and corn syrup from 2001 onwards. If multiple consecutive nest surveys were conducted within a plot, a different area was selected for each survey event so that nests that were disturbed in one survey where not surveyed again at a later date, because Argentine ants frequently abandon nests when disturbed. Nest surveys were usually conducted within all of or half of the 25m by 25m plots (if surveyed once, or twice, respectively), and within randomly selected 10m by 10m quadrats in the larger 100m by 100m plots used in later tests.

1. Initial bait preference test

One of the first priorities for the control program was to find an attractive bait carrier for the toxicant. Bait preference tests have been conducted for the Argentine ant in the lab and in citrus groves in California, and this species has often been found to prefer sugar water over all other baits (Baker et al. 1985, Gaston and Baker 1984). Additionally, Forschler and Evans (1994) found that the commercially formulated Maxforce Granular Ant Bait, hydramethylnon toxicant in a silkworm high-protein bait, was attractive to and effective against the Argentine ant in urban situations in Georgia. Because the sites of infestation in HALE consist largely of undisturbed natural areas, however, regular food sources are less predictable. In a year-long test from 1994 to 1995, eight baits were compared within each of the two HALE ant populations for attractiveness, with feasibility for large-scale control being an important criterion . The eight baits, developed and provided by the Clorox Corporation, were: 1) the Maxforce Granular Ant Bait carrier (minus toxicant), a silkworm protein-based bait with additives, 2) honey granules, a 100% carbohydrate granular bait formulated from Domino Qwik-Flo Honey, 3) insect protein granules, a granular bait consisting of ground silkworm pupae, 4) honey doughy bait, a 100% carbohydrate bait with a doughy consistency, 5) high protein doughy bait, a 100% protein bait with a doughy consistency, 6) fish protein bait, a doughy bait consisting of fishmeal in a gel matrix, 7) sugar water, a solution of 25% pure granulated cane sugar, 8) water.

At both sites, the bait carrier for Maxforce was more popular than any other solid bait. Sugar water was also found to be highly attractive, but at the time was not judged to be practical for large-scale dispersal. There was seasonality in the attractiveness of most baits tested. Maxforce was the most attractive bait in most months, but sugar water was consumed in greater quantities in the fall to early winter. These results are reported in detail in Krushelnycky and Reimer (1998a).

2. 1995 eradication plots

Following the year-long bait preference test at HALE, 14 small (25 m by 25 m) plots were established within the lower ant population to test the effectiveness of Maxforce bait for the purposes of eradication. We felt relatively comfortable using Maxforce bait in the park because its active ingredient, the toxicant hydramethylnon (formulated at 0.9% by weight), has a number of characteristics that minimize adverse effects. It has low acute toxicity towards birds and mammals (EPA 1998), it degrades very rapidly through photolysis (aqueous photolysis half-life is < 1 hour to 3.5 hour [Mallipudi et al. 1986, Chakraborty et al. 1993]; soil photolysis is biphasic with first half-life at 4 days and second at 30 days [EPA 1998]; in daylight photolysis half-life is roughly 12 hours when formulated in Amdro bait granules [Vander Meer et al. 1982]), is not taken up by plants (Bacey 2000), has low solubility in water, and does not leach easily from soil (EPA 1998). When formulated in bait granules and broadcast in a pasture subject to foraging by red imported fire ants, it was undetectable in soil cores within 24 to 48 hours (Apperson et al. 1984).

The 14 plots tested several different treatments:

Maxforce broadcast at 2 lbs/acre (3 replicate plots)
Maxforce broadcast at 4 lbs/acre (3 replicate plots)
Maxforce formulated with hydramethylnon at 0.5% by weight (instead of the normal 0.9%), and broadcast at 4 lbs/acre (1 plot)
Maxforce formulated with hydramethylnon at 0.5% and a different solvent, and broadcast at 4 lbs/acre (1 plot)
Maxforce distributed in 25 equally distributed uncovered piles, at 4 lbs/acre (1 plot)
Maxforce distributed in 25 equally distributed covered piles, at 4 lbs/acre (1 plot)
Maxforce and a honey granule mix, broadcast at 4 lbs/acre (1 plot)
No treatment control (3 replicate plots)

Taken together, these treatments were aimed at testing different application rates (2 vs. 4 lbs/acre), differences in potential toxicant repellency (by testing lower active ingredient concentration and different solvent), differences in bait availability (broadcast vs. piles), and differences in bait attractant composition (the addition of honey granules because these were also found to be fairly attractive in the prior bait preference test). In all of these plots, bait was distributed in the late afternoon in an effort to minimize immediate degradation of the hydramethylnon active ingredient through photolysis.

While all of these treatments yielded very high reductions in numbers of foraging worker ants (over 95%), unfortunately none successfully achieved eradication. Nest surveys indicated that even within the small plots, a substantial number of nests survived with either queens, brood (eggs, larvae, pupae), or both. A second application of the broadcast treatments one month after the first reduced the number of surviving nests, but still failed to eradicate all nests. This failure was believed to result from a combination of several factors. First, the bait has a short active lifespan (probably only several days, due to degradation of active ingredient and molding of bait carrier) and causes mortality quickly (within 24 to 48 hrs). Second, the bait was probably insufficiently attractive to be quickly distributed to all individuals in all nests. The key, therefore, was to find a bait and toxicant combination that ensured that all reproductive individuals received a lethal dose before either mass mortality in the nests ensued or before the bait or active ingredient became inactive or unattractive. These results are reported in detail in Krushelnycky and Reimer (1998b).

3. 1996-97 eradication plots

Despite the less-than-perfect performance of Maxforce bait, we decided at the completion of the 1995 plots to investigate several potential mitigating factors to the bait's effectiveness. These included timing of application (both time of day and time of year), and the effect of ant density in different habitat types and elevations. We also wanted to test several new products: Grants Kills Ants bait stations (arsenic active ingredient), and a new bait, named Maxforce FC, which consisted of the regular Maxforce granular bait carrier formulated with 0.003% fipronil. We chose to not establish replicate plots, but to instead conduct quick experiments that could be followed up with more rigorous tests should initial results look promising.

We established a total of 12 new 25m by 25m plots:

Maxforce broadcast at 2 lbs/acre in the morning in July 1996 at 7300 ft elevation (lower population)
Maxforce broadcast at 8 lbs/acre in the morning in July 1996 at 7300 ft elevation
Grants Kills Ants bait stations (25 stations, one every 5 m) in July 1996 at 7300 ft elevation
Maxforce FC broadcast at 3.3 lbs/acre in the morning in August 1996
Untreated control for above plots

Maxforce broadcast at 2 lbs/acre in the morning in July 1996 at 9200 ft elevation (upper population)
Maxforce broadcast at 4 lbs/acre in the morning in July 1996 at 9200 ft elevation
Untreated control for above plots

Maxforce broadcast at 2 lbs/acre in the morning in July 1996 at 7400 ft elevation at crater floor (upper population)
Untreated control for above plot

Maxforce broadcast at 4 lbs/acre in the morning in February 1997 at 7300 ft elevation (lower population)
Untreated control for above plot

All of the standard Maxforce (with hydramethylnon) plots yielded results very similar to those obtained in 1995. All plots, including the plot treated at 8 lbs/acre, dramatically reduced numbers of workers but failed to kill all nests. We therefore concluded that time of day, season and habitat differences appear to have no great impact on Maxforce effectiveness.

In contrast, the Grant Kills Ants bait stations had very little impact on ant numbers, but the Maxforce FC appeared to be somewhat more effective than standard Maxforce. Numbers of foraging workers were reduced even further, and fewer nests survived. However, given that several nests did persist within the small plot, extrapolation to larger areas would mean that many nests would survive and probably begin rebounding within several years after treatment. It was nevertheless decided to investigate Maxforce FC further, as it yielded the most promising results to date.

4. 1998 eradication plots

In the winter/spring of 1998, we set up five new plots to further test Maxforce FC bait, as well as an experimental sugar water liquid bait formulated with fipronil (this bait was under development by Clorox at the time, but as far as we know it never went into commercial production). Plots were located in the lower population. Each plot receiving granular bait was 100 m by 100 m in size (1 ha in area). We increased the plot size from the previous 25 m by 25 m dimensions to allow for more confident interpretation of results within the plots; with larger plots, it takes ants longer to reinvade from the plot perimeter, and therefore decreases the chances of this movement into the plots confounding the treatment results. The plot testing the sugar water bait was only 50 m by 50 m, however, both because of the large number of liquid bait stations required as plot size increases, as well as the fact that this bait would be replenished in the bait stations throughout the course of the experiment. It was reasoned that the constant presence of bait should prevent reinvasion of the plot from its periphery. Because the larger plots were much more labor intensive, we did not use replicate plots for each treatment (the provisions of Experimental Use Permits in Hawaii, limiting experiments to a total of 10 acres, also prevent replication when using large plots for multiple treatments). In our view this tradeoff was justified, because we felt that it was more important to obtain reliable results for a particular treatment in one plot than more questionable results in multiple replicate plots. Furthermore, treatments yielding excellent results could always be replicated and investigated in greater depth at a later date.

We timed this set of treatments to occur during the estimated period of Argentine ant queen reproduction. Both lab and field work in other locations suggest that Argentine ants only produce queens once a year, with this cohort preceded by a queen execution event in which workers kill roughly 90% of the pre-existing old queens. At this point in time, adult queen numbers should be very low, and this may represent a period of unusual vulnerability for the colony. Prior field observations suggested that new queen production occurs at HALE around April. The first three treatments below were timed to span the period leading up to and including new queen production. The fourth treatment explored the effect of applying baits only during the period when new queen production is thought to occur, applying the same amount of bait as other granular treatments, but concentrated during a shorter time frame.

The treatments tested were as follows:

The first treatment tested a 20% sucrose solution formulated with 0.0005% (5 ppm) fipronil, plus preservatives. Thirty ml of this bait was offered in each of 36 bait stations, spaced every 10 m throughout the 50 m by 50 m plot. Bait stations were first filled on 24 Feb 1998. Because this formulation appeared repellent, it was removed from the plot in March and was replaced with a new formulation without preservatives beginning on 14 Apr 1998.

The second treatment tested the effects of four consecutive broadcast applications, roughly one month apart, of the Maxforce FC granular bait (standard Maxforce granules formulated with 0.003% fipronil) in a 100 m by 100 m plot. The applications were made on 24 Feb, 24 Mar, 21 Apr, and 19 May 1998, each at the rate of 1 lb/acre.

The third treatment was similar to the second in terms of timing and rates of applications, but used two different toxicants. Four broadcast applications were made in a 100 m by 100 m plot, on the same dates as above, but the first application used standard Maxforce Granular Ant Bait (formulated with hydramethylnon), followed by three applications with the Maxforce FC granular bait (all applications were also at a rate of 1 lb/acre).

The fourth treatment was intended to intensively target the period of new queen production. Maxforce FC bait was broadcast at 1 lb/acre once a week for four consecutive weeks from 7 Apr to 28 Apr 1998, in a 100 m by 100 m plot.

The fifth plot received no treatment and served as a control.

The results indicated that the sugar water bait did not work at all (Appendix 2, Figure 1). Initial poor results could be explained by an apparent repellency of the initial formulation of the bait. Because the concentration of fipronil in this bait was extremely low, it is unlikely that ants could detect the toxicant, and the repellency may have been to the preservatives used. A new formulation using no preservatives was offered in April 1998, and initial foraging to these baits was heavy. However, almost no ants could be seen entering the bait stations after several weeks, despite the fact that many ants persisted inside the plot. The bait may have caused rapid mortality among a percentage of the workers, and this in turn may have limited recruitment, or caused the bait to be regarded as toxic or unattractive, although, to our knowledge, such negative conditioning towards toxic baits has not been demonstrated to occur in ants (Josens et al. 2009).

The plot in which Maxforce FC was applied more frequently (weekly), and later in the season, also yielded relatively poor results. Although numbers of foraging ants at bait monitoring stations essentially dropped to zero after the second application (Appendix 2, Figure 2), many nests remained active, and many queens survived, even after the fourth application (not shown). As with previous trials using standard Maxforce baits, these results emphasize the importance of using nest monitoring as a supplement to bait card monitoring, if possible. Over the short term, bait card monitoring did not indicate the high degree of survival evident when inspecting nesting sites. Although regular monitoring was discontinued in this plot after the fourth application, it was subsequently checked periodically to confirm that substantial nest survival remained.

It may be that the timing of the delayed weekly applications was less than ideal, possibly coming just as the new cohort of queens was emerging, rather than when they were still larvae. This application regimen may have been more successful had it been done in March instead of April. Alternatively, the short interval between bait applications may have led to a high degree of bait shyness, where surviving ants learned to avoid the bait on subsequent applications, as mentioned above.

The two plots in which Maxforce was applied over an extended period on a monthly basis yielded better, but somewhat inconsistent results. Both treatments resulted in virtually no ants at monitoring bait cards several weeks after the first application (Appendix 2, Figure 3). However, the treatment which used four applications of Maxforce FC, resulted in no queens observed and only several nests with small numbers of larvae in monitoring quadrats after the fourth application (not shown). In comparison, the Maxforce treatment that included one application of hydramethylnon followed by three of fipronil had more surviving nests after the fourth application. Monitoring in this latter plot was discontinued shortly after the fourth application because of the relatively high number of surviving nests.

In summary, multiple repeated applications with a fipronil-based Maxforce granular bait may achieve results that approach eradication. However, the plot treated with only three applications of this bait (preceded by treatment with standard Maxforce) had more surviving nests, suggesting that at least four applications are necessary, or perhaps that results will be inconsistent in different areas. In addition, the interval between applications may be very important. Applications spaced one month apart yielded much better results than applications spaced one week apart, although differences in the timing of these applications (i.e. time of year) may have also played a role. Further research with this formulation is not currently possible, as Maxforce FC was apparently only produced for several years, and is no longer available.

5. 2001-2002 eradication plots

After a several year hiatus, USGS renewed testing of baits in experimental plots to assess potential for eradication. In 2001-2002, plots were established to test the effectiveness of insect growth regulators (IGRs). In contrast to the acute toxicants hydramethylnon and fipronil, IGRs are compounds that alter the development of insects. The active compounds in most IGR products are analogues of juvenile growth hormone, which controls normal molting of larvae and eclosion of pupae. Because they function by halting development and reproduction, and do not directly cause mortality, insect populations show a delayed response to exposure to IGRs. This type of delayed response is generally desirable in ant control, since it allows foraging workers to exchange baits between other workers, larvae, and queens.

A myriad of IGR bait products are available for ant control, most of these using the active ingredients methoprene, pyriproxyfen, and fenoxycarb. Several granular bait formulations are available using these active ingredients, but the majority of them are formulated for fire ants, using a bait carrier of corn grit and soybean oil. Argentine ant populations at HALE are generally not attracted to these oily formulations. Although to our knowledge, granular protein-based IGR baits were not available, Pharorid, a product marketed primarily for the Pharaoh ant but also labeled for the Argentine ant, was a liquid methoprene (4.9%) product labeled for use with a bait matrix determined and designed by the user. This flexibility can be an advantage when dealing with ant populations that are not attracted to most outdoor bait products.

Three 100 m by 100 m plots were established within the lower ant population. The first plot received two applications of Pharorid Ant Growth Regulator, the second plot received two applications of Pharorid Ant Growth Regulator followed by a single application of Maxforce Granular Insect Bait (with hydramethylnon), and the third plot served as a control. The Pharorid product was combined with a liquid bait consisting of a 6:4 mixture of blended canned tuna and corn syrup, which was known to be attractive to HALE Argentine ants. Pharorid was combined with this mixture at the label rate of 1mL Pharorid to 20g bait, resulting in a final concentration of approximately 0.25% methoprene. The Pharorid/liquid bait was applied in bait stations deployed in a 5 m grid pattern, for a total of 441 stations in each plot, with each station containing 9.1 g of the bait mixture to match the recommended application rate of methoprene for indoor use. Both treatment plots were treated with Pharorid on 12 Nov 2001 and 5 Jan 2002, and Maxforce was broadcast at 1.5 lbs/acre in the second plot on 6 Feb 2002. Because IGRs can work relatively slowly, plots were monitored until Nov 2002.

Results from foraging ant monitoring and nest surveys indicated that while ants foraged heavily to the Pharorid bait stations, this IGR treatment did not cause much, if any, ant mortality. Foraging worker numbers were not substantially different from the control plot on most monitoring days (Appendix 3, Fig. 1). More importantly, nest surveys indicated that brood production did not appear to be impacted, even after one year (not shown). In the plot where Pharorid applications were followed by a Maxforce application, results prior to the Maxforce application were similar to those in the Pharorid plot. After Maxforce application, results were qualitatively similar to those obtained in previous Maxforce plots, but perhaps slightly more effective quantitatively; foraging worker numbers were greatly reduced compared to the control (Appendix 3, Fig. 2), and nests were significantly impacted, but at least a few nests survived.

It is not clear why the methoprene IGR (Pharorid) failed to have a stronger impact. Rats interfered with some of the bait stations, especially during the first application, however this did not appear to occur until several days after the stations were first placed in the field, allowing ants sufficient time to consume the bait. A second factor may have been that the spacing of the bait stations was less dense than that recommended for indoor use, due to practical limitations with the use of bait stations. Nevertheless, the total dosage of methoprene per area was equivalent to the recommended rate for indoor use, and the maximum distance to the nearest station for any nest within the plot was only 3.5 m, which is well within normal foraging distances for Argentine ants. Neither of these explanations therefore seems likely to account for Pharorid’s ineffectiveness.

6. 2002-2003 bait preference trial and eradication plots

In Apr 2002, we conducted a bait preference trial using several products that had either recently appeared on the market or had never been formally tested at HALE, and then tested one of these in experimental eradication plots. The baits compared in the preference test included: Advance

Granular Carpenter Ant Bait (Advance GCAB), Advance Granular Ant Bait (a product similar to Advance Granular Carpenter Ant Bait, but with a finer granule size and presumably different ingredients), CB-441 Granular Ant Bait with IGR, Distance Fire Ant Bait, Maxforce Granular Insect Bait (GIB), an unformulated protein paste, Siege-Pro Fire Ant Bait, and “Tast-E-Bait”, an unformulated experimental carbohydrate bait. Small pre-weighed petri dishes, each filled with 1 g of bait product, were exposed to Argentine ant workers for 24h and then reweighed. The trial was conducted in the lower ant population at HALE, on a 150 m transect with stations placed 10m apart, for a total of 16 stations. All eight baits were offered at each station. In order to adjust for weight changes due to desiccation or absorption of moisture, a complementary set of stations was deployed in which ants were excluded with Tanglefoot.

Advance GCAB was highly preferred over other bait products, including the currently used Maxforce GIB; by weight, ants removed over 80 times more Advance than Maxforce (Appendix 4, Table 1). When a small amount was spilled on the ground, workers responded quickly to the bait, becoming visibly excited and eagerly gathering bait granules. The bait matrix of Advance GCAB is the standard corn grit saturated with an oil, although the type of oil is unknown and other proprietary additives may be included. Because of the complete lack of appeal of other corn grit/oil-based baits (including those tested here, such as Distance Fire Ant Bait and Seige Pro Fire Ant Bait, which use soybean oil), we assume that the attractiveness of Advance GCAB is due either to the use of a different type of oil or to additives. We had not previously found any granular bait to be nearly as attractive as Advance GCAB, so we decided to test this bait in experimental field plots.

The active ingredient of Advance GCAB is 0.011% abamectin, a neurotoxin derived from a bacterium, *Streptomyces avermitilis*. The product label for Advance GCAB also reports that it can eliminate colonies by halting egg production, but we could find no reports in the literature of queen sterilization in the Argentine ant. Although the bait is primarily marketed for use against carpenter ants, it is also labeled for use against Argentine, bigheaded, crazy, field, fire, little black, pavement, pharaoh, odorous house, and thief ants.

Three 100 m by 100 m plots were established within the lower Argentine ant population at HALE. The three plots were randomly assigned to treatments: one plot was designated as the control plot, the second plot received two applications of Maxforce GIB, and the third plot received two applications of Advance GCAB. The first broadcast application for both treated plots was conducted on 7 Oct 2002, followed by a second application in both plots two weeks later on 21 Oct 2002.

Although Advance GCAB was much more attractive to Argentine ant workers at HALE than the currently used Maxforce GIB, Advance was not nearly as effective in controlling the Argentine ant in experimental plots. Despite low numbers of workers visiting bait monitoring cards during the months of October and November after Advance GCAB broadcast (Appendix 4, Figure 1), workers were observed in high densities on the ground in the plot during this period. It therefore appears that low numbers on bait cards during this period did not represent a population crash, but rather a shift in foraging behavior or preference. In contrast, Maxforce GIB caused an immediate crash in numbers of workers, and numbers remained very low at least 9 months after initial treatment (Appendix 4, Figure 2).

Nest surveys corroborated the bait card monitoring data. Numbers of workers in nests in the plot treated with Advance GCAB remained high, and roughly followed the same trends as numbers in the control plot (not shown). Queens, larvae and pupae were also continually present in the Advance plot throughout the survey period, in numbers comparable to or greater than numbers in the control plot. This bait therefore apparently did not sterilize queens. In contrast to the Advance GCAB plot, workers, queens, larvae and pupae were all much lower in the plot that received two applications of Maxforce GIB, relative to the control plot. This was especially true in November, a month following the second application, when almost no ants were seen during under-rock surveys. In Mar 2003, ant numbers in the Maxforce plot were higher than in November, but numbers of workers were still very much lower than in the control plot or in the Advance plot. These results were typical for Maxforce GIB.

It is unclear why Advance GCAB was so ineffective. One possible explanation is that Argentine ant workers collected the Advance GCAB granules, but either the granules were dropped before workers reached the nest, or the granules were not consumed by ants in the nest. Since Argentine ant workers are usually not highly attracted to corn grit and (soybean) oil formulations, chemical additives may be responsible for the attractiveness of Advance GCAB. If these additives are pheromone analogs or other behavior-altering substances rather than food-based attractants, they may induce worker ants to retrieve bait, but might not induce them to feed on it, especially if it is not a preferred food item.

Insufficient sharing of Advance GCAB between nestmates could also be caused by rapid mortality of foraging workers exposed to the abamectin toxicant. Within 20 minutes of offering the baits in the initial bait acceptance trials, ants were seen visiting bait stations in very high numbers, but upon revisiting the stations after 24h, very few live ants were seen in the stations or on the ground nearby, despite significant amounts of Advance GCAB remaining in many stations. Many dead ants were found inside the bait stations. Although ants were exposed to several different baits with different active ingredients, Advance GCAB was the only bait removed in significant quantities, so it is possible that abamectin was responsible for this apparent rapid mortality. Hooper-Bui and Rust (2000) found that abamectin caused substantial kill of workers in laboratory colonies exposed for 24 hrs, but not more so than hydramethylnon, boric acid or fipronil.

7. 2004-2005 eradication plots

Because of the inadequate effectiveness of all of the granular baits tested at HALE to date, we decided to begin investigating liquid baits in 2004, despite the greatly increased labor and cost involved. Our rationale was that identifying an effective bait product would be highly valuable even if a realistic delivery system was not immediately obvious; work on the second component of the challenge, developing a feasible method for bait delivery, could be pursued if an appropriate bait was found.

Although liquid baits are more difficult to apply than granular baits, usually requiring some type of bait station to house the bait, they can have certain advantages over granular products. First, most liquid baits use sugar water as their base, which is highly attractive to a wide variety of invasive and pest ant species (Klotz and Moss 1996, Klotz et al. 1996, Klotz et al. 1997a, Klotz et al. 1997b, Klotz et al. 1998, Ulloa-Chacon and Jaramillo 2003). Second, because they are ingested and transported internally, liquid baits are easily retrieved and shared among nestmates through trophallaxis. Third, liquid baits in bait stations can be monitored and replenished to provide longer and more thorough access for the target ants (while limiting access for non-target species). Finally, a number of commercial liquid baits are formulated with borate toxicants, which tend to be regarded as relatively benign pesticides (e.g., see below). Boric acid has been used to suppress ants in homes for decades (Klotz et al. 1997b), and recent laboratory and field studies suggest that when formulated at low concentrations (0.5-1%) in sugar water baits, boric acid can be as effective or more effective against certain ants than more recently developed toxicants (e.g. Hooper-Bui and Rust 2000). For all of these reasons, there has been renewed interest in liquid baits formulated with boric acid or other borates for use in certain agricultural and urban settings (Klotz et al. 1998, Daane et al. 2006, Greenberg et al. 2006, Nelson and Daane 2007), and these same features also make these baits an attractive potential option for ant control in sensitive natural area situations.

We decided to investigate the effectiveness of a simple 25% sucrose solution formulated with 0.5% boric acid. Commercial products using boric acid that are registered for outdoor use typically contain 5% to 7% boric acid, however recent laboratory research indicates that much lower concentrations, in the range of 0.5–1% boric acid, are more effective against Argentine ants (Hooper-Bui and Rust 2000). We also included a treatment using Maxforce Granular Ant Bait, both as a standard against which to judge the efficacy of the boric acid bait, and in order to test an application protocol not previously assessed: the Maxforce plot was treated at the label rate approximately once every five weeks, for a total of four applications. This treatment time interval was judged to be most effective in targeting newly emerged larvae and adults that may have been protected from exposure to previous treatments in their non-feeding egg or pupal stages. In comparison, the liquid boric acid bait would be present and available continuously.

Three plots were established within the lower Argentine ant population in the west slope shrubland area, between 2100 and 2250 m elevation. One plot (50 m by 50 m) tested the boric acid bait, one plot (100 m by 100 m) tested the Maxforce bait, and one plot (50 m by 50 m) was not treated and served as a control. We placed boric acid bait stations every 10 m in a grid pattern within the boric acid plot, for a total of 36 bait station locations in the plot. The boric acid bait stations were placed in the field in late July 2004, and were refilled with fresh bait approximately every five weeks through February 2005. Maxforce bait was applied in the Maxforce plot on July 21, 2004, followed by three subsequent broadcast applications on August 27, 2004, October 6, 2004 and November 4, 2004. The granular bait was applied with hand spreaders at the label application rate of 1.5 lbs/acre.

Mean number of foraging ants at bait card monitoring stations failed to decrease after the first Maxforce treatment (Appendix 5, Fig. 1). This was highly unusual; all previous treatments using Maxforce in the park have resulted in immediate (1-3 days post treatment) and dramatic (>90%) reductions in foraging ant numbers. Ant numbers decreased substantially after the second treatment, and continued to decrease after the third and fourth treatments (Appendix 5, Fig. 1), but it was not until December of 2004, after the fourth treatment, that foraging ant numbers declined to a level that would normally be achieved with one treatment.

Despite this eventual decrease in foraging ant numbers, nest surveys indicated that the Maxforce treatment was relatively ineffective. The number of reproductive nests per quadrat only decreased slightly over the course of the four treatments, similar to the control plot (Appendix 5, Fig. 2). Within nests, the average worker larval abundance increased over the course of the four treatments, also similar to the control plot (Appendix 5, Fig. 3). Likewise, total number of queens per quadrat showed a trend similar to that of the control plot (Appendix 5, Fig. 4), indicating little or no effect resulting from the Maxforce treatments. These unusual and disappointing results seem likely to have involved a change in the Maxforce bait formulation. The Maxforce granular bait received in 2004 was noticeably smaller in granule size than bait used in the past. Smaller granules may degrade faster when broadcast, and smaller granules may affect foraging ant behavior in ways we do not understand. In addition, the smaller granules may be easier to ingest by workers than the previous larger granules. This may result in workers being targeted more directly, and therefore dying before they are able to share much of the bait with the brood and queens. We also have reason to believe that the bait matrix has varied significantly in quality over time, which may have severely impacted its effectiveness.

In the boric acid plot, mean number of foraging ants at bait card monitoring stations decreased slowly, over the course of about four months (Appendix 5, Fig. 1). We expected the boric acid bait to work relatively slowly, both due to its mode of action and the low concentration of our formulation. This rate of decrease was slower than expected, however. Nest surveys also indicated a slow, but consistent, increase in mortality. The total number of reproductive nests per quadrat decreased over time, particularly by January and February of 2005 (Appendix 5, Fig. 2). Part of this decrease undoubtedly resulted from the same factor that caused number of nests to decrease in the control plot – the fusing of nests into fewer, larger nests in the late fall and winter. The remaining reproductive nests in the boric acid plot, however, had substantially fewer worker larvae than the control plot (Appendix 5, Fig. 3), fewer queens (Appendix 5, Fig. 4), and egg production appeared to have ceased (Appendix 5, Fig. 5). Although the failure to observe any queens or eggs in February of 2005 could indicate that the nests were all on the path to eventual collapse, eggs, which were scarce in the control plot at this time of year, are easy to overlook, and queens are also easy to miss when there are only a few of them. Moreover, there were a few nests in the plot with substantial amounts (50-100) of small larvae, indicating that they had been recently produced.

While eradication was not achieved in the boric acid plot, most nests appeared to have died and the remaining survivors were mostly small remnants. Surviving nests were not located further from boric acid bait stations than dead or nonreproductive nests (two-sample t tests on both the entire post-treatment data set and a reduced data set including only the last three monitoring events were not significant; $t = -0.48$, $P = 0.634$, and $t = 0.82$, $P = 0.423$, respectively), suggesting that the 10 m spacing interval of the stations was not too large and was not the reason eradication did not occur. Encouragingly, ants continued to feed on the boric acid bait throughout the experiment, indicating that they were unaware of its toxicity and continued to find it attractive.

One problem with the current design was the plot size. We did not anticipate this to be a problem; we reasoned that since the boric acid bait would be available continuously, it would continuously kill any new nests that attempted to move in from the periphery of the plot. But because the boric acid worked so slowly, it is possible that new nests could have moved quite far into the plot before they were significantly impacted. Another potential problem concerns the ability of Argentine ant workers to rear new queens from young larvae. This, combined with an almost continuous presence of haploid eggs in the nest (which if raised become males), allows nest fragments to potentially produce new fully reproductive nests (Aron 2001). The slow acting nature of the boric acid bait may therefore make it difficult to eradicate all nests, since nests that decline gradually over long periods may be commonly triggered to produce new queens.

Despite these limitations, we felt that the results with the boric acid sugar water bait were encouraging, and that liquid baits formulated with borates could profitably be investigated further.

8. 2007 Gourmet Liquid Ant Bait eradication plots

More extensive studies of liquid baits formulated with borates were initiated in 2007 with an investigation of Gourmet Liquid Ant Bait. Gourmet Liquid Ant Bait, manufactured by Innovative Pest Control Products, uses a mixture of several different sugars as attractants and the borate DOT (disodium octoborate tetrahydrate) for the active ingredient (at 1% concentration). We chose to test this bait among commercially available sugar water based baits for several reasons. First, Gourmet was previously found to be both attractive to and effective against Argentine ants in California (Greenberg et al. 2006). Second, the Gourmet label includes language permitting the user to dilute the bait with any food grade products that might increase bait attractiveness. The borate concentration in the bait can also be decreased in this way. Finally, Gourmet Liquid Ant Bait is registered for agricultural use (in approved bait stations), and Innovative Pest Control Products has obtained a Special Local Need label amendment allowing Gourmet to be used in organic fruit and nut orchards in California. The flexibility afforded by these types of provisions could be very important in gaining approval for other use patterns that involve sensitive habitat types. Importantly, the owner of Innovative Pest Control Products expressed interest in working towards registration of wider use patterns in Hawaii should results be favorable.

Three main areas of research relevant to testing Gourmet Liquid Ant Bait at HALE were pursued: the selection of an appropriate bait station design; work aimed at improving attractiveness and consumption of bait formulations; and testing the efficacy of the bait for eradicating Argentine ants in experimental plots. This work is summarized briefly below; a full report is provided in Appendix 6.

After evaluation of a commercial bait station, Km AntPro, we decided that a home-made bait station design was better suited for the situation at HALE. This bait station consisted of an outer case made from pvc tubing and end caps, and an inner tube that housed the liquid bait. Although the commercial bait station was cheaper per unit volume of bait, the home-made pvc station was much cheaper per bait station, which was deemed preferable for HALE where more bait stations each holding less bait is likely to be most effective. Moreover, the home-made station was easier to use in uneven terrain, less subject to evaporative water loss, and better at excluding non-target arthropods.

There was a surprising amount of difficulty with attractiveness of the Gourmet bait. We investigated a number of modifications to the bait formulation in an attempt to improve attractiveness, including addition of protein (in the form of hydrolyzed casein), dilution with water and sugar water, adjustment of pH with sodium bicarbonate, and finally dilution with different fruit juices. Favorable results in bait preference tests (e.g. Figs. 3 and 6 in Appendix 6), however, did not translate into high levels of attractiveness or consumption in the experimental ant control plots. This was true for two different formulations that were tested in the plots consecutively (in an effort to improve bait performance): 75% Gourmet + 5% casein, diluted with 25% sugar water, initially, and 50% Gourmet + 5% casein + 45% grape juice, subsequently.

Because of the low attractiveness of the bait formulations, there was a low level of ant control over the course of 32 weeks in the two 1.68 ha experimental treatment plots that were situated on an ant population boundary at HALE. The two plots tested two different bait application rates: stations placed in either a 10 m grid pattern or a 20 m grid pattern within the plots. Foraging ant numbers in both treatment plots appeared to be impacted only slightly (Appendix 6, Fig. 8), and eradication was clearly not achieved: reproductive nests, which possessed queens, eggs and worker larvae, persisted throughout the experiment (Appendix 6, Figs. 9-12). Finally, the Gourmet treatments did not prevent outward spread of the ant population boundary. Ants spread furthest from the control (no treatment) plot (mean of 39.6 m from May to December 2007), but not significantly more so than from the 10 m grid treatment plot (mean of 29.7 m) (Appendix 6, Fig. 13).

Despite the poor results in this study, we believe that sugar water based liquid baits should be tested in other locations and with other invasive ant species because of the well known importance and attractiveness of liquid carbohydrate food sources for invasive ants in general. Feeding preferences of ants can be variable both spatially and temporally, and liquid baits may prove to be an important control tool for certain use patterns, especially where other baits are prohibited. Moreover, other sugar water based liquid baits could perform better than Gourmet at HALE, and could be explored for control in smaller but high risk areas like greenhouses or cabins.

9. 2007 0.5 HP Granular Ant Bait eradication plots

In 2007 we learned of a new granular bait in development by Sumitomo Chemical Australia, provisionally named 0.5 HP Granular Ant Bait. This bait incorporates several features that are designed to make it attractive to and effective against a variety of invasive ants. Like several currently available fire ant baits, the bait is formulated with two active ingredients – a combination of 0.35% hydramethylnon and 0.25% pyriproxyfen. Hydramethylnon is a metabolic inhibitor, and is the same toxicant used in Maxforce Granular Insect Bait, a product tested extensively against Argentine ants at HALE. Pyriproxyfen is an insect growth regulator aimed at halting development of immature stages and sterilizing queens. This combination of hydramethylnon with an insect growth regulator has been employed in the campaign to eradicate red imported fire ants (*Solenopsis invicta*) in Australia. In addition to using two active ingredients, 0.5 HP Ant Bait is unique in that it combines two different bait carriers – a protein granule and a corn grit granule soaked in soybean oil. Each of these granule types has been commonly used separately in individual ant bait products, but 0.5 HP Ant Bait blends the two types together in a single bait with the goal of improving attractiveness and consumption for a wider variety of pest ant species. The protein granule is composed of fish meal, and has been used as the bait carrier (with a different active ingredient) in yellow crazy ant (*Anoplolepis gracilipes*) control work on Christmas Island. The corn grit/soybean oil granule is similar to that used in a wide variety of baits targeting fire ants, but has been augmented with a proprietary ingredient to improve attractiveness to species, like the Argentine ant, that typically aren't strongly attracted to corn grit/soybean oil based baits. The combination of both granule types may increase effectiveness if bait preferences vary among nests either spatially or temporally (if multiple applications are made).

We decided to test this new granular bait in experimental plots at HALE in the summer to fall of 2007. This experiment is summarized briefly below; a full report is provided in Appendix 7.

We established three 1 ha treatment plots, plus a fourth 1 ha control plot, to test three bait treatments: corn granules only, protein granules only, and the two granules blended together as intended in the commercial product. Each treatment received two applications of granules, separated by five to six weeks, at an application rate of 2.24 kg/ha (2 lbs/acre). In addition, excess bait permitted a third application of only the central 30 m by 30 m portions of the corn granule and protein granule treatments (but not the blend treatment).

Numbers of ants recruited to baited monitoring cards were strongly reduced after the first and second applications in all three treatments, but never reached 0 in any plots (Appendix 7, Fig. 2). Incursion of ants into the plots from the periphery was apparent after the first application, but reached less than 25 m into the plots and did not appear to occur after the second application (Appendix 7, Fig. 3). Nest surveys confirmed the continued presence of active, reproductive nests or nest fragments in the central portions of all three treatment plots throughout the experiment and up to 19 weeks after the initial application (Appendix 7, Figs. 4-7). These surviving nests contained eggs and young larvae, suggesting low effectiveness of the insect growth regulator component of the bait (pyriproxyfen), at least under this application protocol. Both baitcard monitoring and nest survey monitoring therefore indicated that eradication did not occur in any of the treated plots, even after two to three applications of 0.5 HP Ant Bait. It was concluded that this product, if used alone, is unlikely to eradicate Argentine ants at HALE. It may, however, be a useful tool in combination with other effective products. Moreover, it produced results comparable to those observed with other bait products formulated with hydramethylnon at concentrations two to three times higher. In this respect, it could become a preferred product for species known to be effectively controlled with hydramethylnon, such as the big-headed ant and (in some situations) the little fire ant.

10. 2008 Advion Insect Granule eradication plots

Advion Insect Granule (IG) is a granular bait produced by DuPont, and consists of a proprietary bait carrier formulated with 0.22% of the active ingredient indoxacarb. Indoxacarb is a relatively new, fast-acting nerve toxicant, and has been found to be effective in controlling fire ants when formulated in DuPont's Advion Fire Ant Bait. Like other fire ant baits, Advion Fire Ant Bait uses a corn grit and soybean oil granular bait carrier that is not very attractive to Argentine ants. Advion IG is currently the only available granular bait formulated with indoxacarb that uses a bait carrier different from the standard corn grit/soybean oil carrier. While Advion IG was originally targeted for mole cricket control (it was previously marketed under the name Advion Mole Cricket Bait), DuPont reports that it has also shown good efficacy against several pest ant species, including Argentine ants in laboratory trials (M. Coffelt, pers. comm.).

We tested two different treatment types using Advion MCB against Argentine ants in experimental field plots at HALE in the summer to fall of 2008. The first treatment consisted of two sequential applications of Advion IG at a rate of 2.24 kg/ha (2 lbs/acre). While the Advion IG label allows application rates of up to 200 lbs/acre in turfgrass, 2 lbs/acre is similar to application rates used for other granular ant baits at HALE, and is more in line with the label application rate of Advion Fire Ant Bait (1.5 lbs/acre). The two applications were separated by about five to six weeks, with the second application intended to target nests and nest fragments that survived the first application, especially individuals that were in the egg or pupal stages at the time of the first application. The second treatment type consisted of one application of Advion IG at a rate of 2.24 kg/ha, followed five to six weeks later by an application of Maxforce Granular Insect Bait (GIB) at a rate of 1.68 kg/ha (1.5 lbs/acre, label rate). Maxforce GIB (formulated with 1.0% hydramethylnon) has been the most commonly used ant bait in efforts to control Argentine ants at HALE. It is fairly attractive to Argentine ants and typically results in high levels of worker ant reduction, although nest fragments always survive. The second treatment type in this experiment was designed to test whether the sequential use of two different baits (including different bait carriers and active ingredients) may increase effectiveness. For example, bait preferences could vary among nests either spatially or temporally, or surviving nests could become "bait shy" and avoid a particular bait after the initial exposure, although this phenomenon has not been previously demonstrated (Josens et al. 2009).

In combination with this field test of Advion IG efficacy, we investigated the attractiveness of Advion IG in the field (relative to Maxforce GIB), and the efficacy of Advion IG for controlling laboratory Argentine ant colonies (also compared to Maxforce GIB). These additional tests were intended to reveal how Advion IG performs at different scales and in different settings, and indicate whether certain tests may reliably predict outcomes of large-scale control efforts. The results of these experiments are reported briefly below; a full report is provided in Appendix 8.

Advion IG caused high mortality of worker ants (>90%) in laboratory colonies in 10 days, although only 33% of queens died in this time period. In comparison, less than 15% of workers died in laboratory colonies offered Maxforce Granular Insect Bait (GIB) in 10 days, and no queens died (Appendix 8, Fig. 10). As reported above, Maxforce GIB has been used on numerous occasions in attempts to eradicate or slow spread of Argentine ants at HALE, typically causes high mortality of worker ants, and therefore serves as a useful standard with which to compare new bait products. The low mortality of workers in laboratory colonies offered Maxforce GIB may have been due in part to higher attractiveness of other protein and carbohydrate food sources that were provided throughout the experiment.

In a field-based bait preference test, Advion IG granules were significantly less attractive than Maxforce GIB granules, with roughly four times as many ants attracted to the Maxforce GIB (Appendix 8, Fig. 11). The relatively low attractiveness of Advion IG was consistent with poor results in 1 ha experimental efficacy plots. In two separate plots, a single broadcast application of Advion IG at 2.24 kg/ha (2 lbs/acre) yielded no reduction in numbers of workers attracted to monitoring bait cards (Appendix 8, Fig. 4). Lower numbers of ants at four and five weeks after application were observed in both treated plots as well as the control plot, suggesting that this fluctuation was due to external factors such as weather. Numerous active, reproductive nests persisted in both treated plots (Appendix 8, Figs. 6-9). A second application of Advion IG five weeks after the first in one of the plots also yielded no discernable reduction in numbers of worker ants in the four weeks following treatment. In the other treated plot, a broadcast application of Maxforce GIB, at 1.68 kg/ha (1.5 lbs/acre, the label rate), five weeks after the initial Advion IG application resulted in a substantial reduction in numbers of workers at monitoring bait cards, similar to results obtained in prior tests with Maxforce GIB (Appendix 8, Fig. 4). Multiple reproductively active nests persisted in both treated plots, however. These results strongly suggest that Advion IG, either alone or in combination with another bait product, is unlikely to yield effective control of Argentine ants in natural area settings in Hawaii, at least under the application protocol tested.

Previous trials of ant control products at HALE have often found that bait preference tests do not accurately predict outcomes in field trials. This was not true in the present study, however tests of Advion IG at different scales yielded completely different results. It is hypothesized that the much higher mortality of workers in laboratory colonies resulted from topical exposure incurred while investigating and contacting the granules and subsequent self-cleaning. The level of incidental exposure of worker ants to granules broadcast in the field is undoubtedly dramatically lower, and may explain the lack of observed mortality in the field plots.

D. Efforts at Argentine ant containment

Early tests of Maxforce Granular Ant Bait provided what appeared at the time to be promising results: a very high level of mortality among worker ants, and mortality of a substantial number of nests in treated areas (e.g. see sections III.C.2-3 above). Although we subsequently realized that we weren’t able to improve on these results and that Maxforce was therefore unlikely to be capable of eradicating Argentine ants at HALE, we hypothesized that the massive reduction in foraging ant numbers after Maxforce application could disrupt normal colony activities, such as dispersal, and therefore slow or halt the Argentine ant’s spread in the park. Such a containment strategy could prove to be very important, preventing the populations from becoming larger while we continued to screen different products and hopefully find one that could eradicate the populations. We therefore initiated work aimed at determining whether annual applications of

Maxforce to the expanding perimeters of the two populations could achieve this secondary goal of containment. These efforts are summarized below.

1. 1996 experimental perimeter plots

In 1996 we established two experimental plots situated along rapidly expanding sections of the two ant populations. The two plots varied greatly in size and were designed for different purposes. The smaller, 120 m by 260 m (2.9 ha) lower population plot, was intended to simulate a small section of what could potentially be a 120 m wide broadcast application encompassing the entire expanding perimeters of both populations. This plot was situated such that the treated area extended 100 m back into the lower ant population and 20 m beyond the population perimeter as a buffer (Appendix 9, Figures 1 and 2). The larger, 20.5 ha upper population plot was designed to test whether treating a much wider perimeter area (up to 390 m wide) would be more effective than the narrower area (120 m wide) tested in the first plot. Bait was applied to both plots aerially, using a bait hopper suspended from a helicopter, at a rate of 2.25 kg/ha on 19 Aug 1996. Monitoring transects perpendicular to the population perimeters were established in treated and adjacent control areas for both plots. These were monitored monthly post-treatment for one year to assess the rate of spread in each area.

Monitoring after treatment indicated success in both plots in halting ant population expansion for one year, with an exception occurring in only one spot in the larger, upper population plot. Meanwhile, adjacent untreated population perimeters advanced 65 m in the lower population and 80 m in the upper population in one year. Foraging ant numbers at bait monitoring stations appeared to recover more slowly in the smaller, lower population plot, suggesting that this treatment design was more effective than the wider perimeter treatment design. We believed this to mainly be a function of the greater ease in achieving uniform aerial coverage in a smaller plot.

Recovery in foraging ant numbers in the perimeter plots, over a one year period, did not appear to result from reinvasion from the rear of the plot, but instead occurred as small, scattered pockets of survival within the plot. This pattern of mortality and survival was consistent with the results obtained in the initial much smaller eradication plots, strongly supporting the earlier conclusions that Maxforce did not yield eradication of ants in the smaller plots and that nests found in the plots after treatment represented surviving ants rather than reinvasion from outside the plots. The pattern of recovery in the perimeter plots also suggested that using a wider border treatment design would not be more effective in slowing spread because recovery occurred predominantly within the treated area, not as recolonization from behind the treated area.

This experiment is described in more detail in Krushelnycky et al. (2004). Because of less extensive monitoring methods for the larger and more remote upper population plot, however, only the smaller lower population perimeter plot is discussed in this paper.

2. 1997-2004 containment efforts

We found the results obtained in the 1996 perimeter plots highly encouraging, and decided to implement the experimental perimeter treatment technique on a larger scale in an effort to completely halt outward spread of the two ant populations. If successful, this method could become a temporary management technique to contain the Argentine ant in the park.

Based on the results of the 1996 perimeter plots, we designed a containment experiment that would treat the entire expanding perimeters of both ant populations with Maxforce Granular Ant Bait. Only actively expanding perimeters would be treated (identified from the 1980 to 1997 patterns of spread analysis), and only a narrow, 120 m wide perimeter treatment would be used. This would minimize the cost of the bait application, would minimize the amount of pesticide used, and based on results in the 1996 plots should not decrease the effectiveness of the technique.

The first perimeter treatment occurred in August 1997, and covered 86 ha on the west slope and in the crater (Appendix 10, Figure 1). Despite some problems, including difficulty for the helicopter pilot to follow the winding treatment area boundaries over very uneven terrain, the application was a general success. Monitoring over the next year at monitoring stations (Appendix 10, Figures 2 and 3) indicated that the treatment was effective in most areas, however a few places appeared to have been missed in the aerial application. Likewise, the ant population boundaries were held in place over most of their peripheries, but spread did occur in a few locations. In general, the treatment was viewed as effective, but less successful than the 1996 experimental plots. This was not surprising, given the greater difficulty of broadcasting the bait evenly over all designated treatment areas.

This experimental perimeter treatment was continued once annually from 1998 to 2004. Halfway through this period, in 2001, the overall effectiveness of the technique in slowing spread was summarized (Haines et al. 2002). This analysis indicated that the annual perimeter treatments had on average slowed the rate of spread in the lower and upper ant populations by 61% and 65%, respectively (Appendix 10, Figure 4). While this was estimated to have prevented 108 ha of the park from being invaded by Argentine ants, it fell far short of the goal of completely containing the two populations. In particular, the perimeter treatment reduced rates of spread by less than 50% in the fastest spreading areas. The technique was still judged to be worthwhile, but in need of improvement.

Each year the border treatment methodology was modified to greater or lesser degree in an attempt to increase effectiveness. These changes included implementing a 30 m buffer zone beyond the population boundaries instead of a 20 m buffer; modifying the methods for mapping population boundaries so that most or all sections of the boundaries were mapped or re-mapped as close to the bait application date as possible; smoothing the treatment area boundaries to make it easier for the pilots to navigate the resultant flight paths; improving and enlarging the flags marking the treatment boundaries to ease the pilot’s job; and incorporating hand treatment of some areas to help smooth the flight paths. Some of these changes were retained, while others were discarded.

Despite these attempts to improve methods, a number of problems remained. While helicopters remained an essential component of the technique, many of the problems centered on their use. Even with our steps to improve visibility of treatment area markers and to make the flight path less erratic, pilots consistently complained that it was difficult to follow the intended flight path.

We were also never able to overcome the challenge of clearly marking the treatment boundaries in an area with many gulches, ridges and steep cliffs. We perceived that enthusiasm for the project among the pilots was often not very high, due mostly to these difficulties. Since the ant project only contracted helicopters once a year, we had little leverage with the helicopter company, and this increased the chances that our operation would get postponed if a more valued contract demanded their services. This, along with the difficulty of coinciding clear, dry weather (necessary for bait application) with available flight dates, made scheduling and completing the operation an extreme challenge. As operation dates got postponed, the ant population perimeters continued to move and threatened to spread beyond the designated treatment areas.

Problems with the perimeter treatment reached a climax in 2004, when we received our shipment of Maxforce ant bait. For reasons that we were unable to elucidate, the granules were much smaller than in previous formulations. We were concerned that the small granules would drift much more than the previous, larger granules, compromising the efficacy of the treatment. Despite much effort, we were unable to find and receive a shipment of larger granules in the short period before our scheduled operation. It appears that Bayer had shifted to a smaller granule size, either consciously or inadvertently, and larger granules were unavailable.

During the aerial treatment operation, the granules did in fact drift more than usual; the pilot, however, was able to alter his flying technique to decrease the magnitude of this problem. But in the course of concurrent small plot eradication trials, it became apparent that the bait was substantially less effective than previous formulations, irrespective of problems with aerial delivery (since bait in the 2004-2005 eradication plot was broadcast with hand spreaders on the ground; see section III.C.7 above). As a consequence, the perimeter treatment that year was largely a failure. Ant numbers at monitoring bait stations actually increased from an average of 9.4 pre-treatment to an average of 16.3 at one to two weeks post treatment; normally, Maxforce bait would have caused a dramatic decline in foraging ant numbers after that interval. The lower effectiveness may have resulted from a different mode of handling and retrieval of the smaller granules by worker ants; alternatively discussions with Bayer suggest that the quality of the granular bait matrix, manufactured overseas, may have varied over time (unbeknownst to the company at the time).

Because we were no longer able to reliably obtain Maxforce bait of the granule size and quality desired, and because of the other problems discussed above, the perimeter treatment was discontinued after the summer of 2004. The technique was not becoming more effective with more experience and the various adjustments that were implemented, and the most recent results did not justify the cost and effort involved. Funding and experienced personnel availability for the project also became more limited during this period, and for all of these reasons we decided that it was better to focus limited time and resources on testing newer baits for eradication potential.

IV. SUMMARY OF MANAGEMENT EFFORTS TO DATE AND PROSPECTS FOR THE FUTURE

Between 1994 and 2009, 30 commercial or experimental (i.e. in development) ant bait products were evaluated to some degree for potential effectiveness against Argentine ants at HALE (Table 1). Twenty of these were only screened for attractiveness to ants at HALE, either in formal bait preference tests or more informally to gauge general attractiveness, and were subsequently rejected because ants were judged to demonstrate insufficient interest in the baits to justify further testing. Ten baits elicited greater interest by ants and were tested in field plots. These included granular, liquid and one solid product, representing a total of at least 7 different bait matrix formulations and 8 different toxicants. In addition, we tested a number of different combinations of these baits, as well as variations in application rates and methods, for a total of 28 different treatment types.

Only a few of the bait products tested yielded what could be considered good results (Table 1). The first of these is Maxforce Granular Ant Bait (also sold as Maxforce Granular Insect Bait in more recent years). Maxforce was the first bait tested in field plots at HALE, and produced promising early results that provided hope that eradication might be achieved with an easily dispersed granular bait. After much additional work with Maxforce, however, it became evident that this bait would never completely kill enough nests to make it a viable option for a large scale eradication attempt. Results were qualitatively similar regardless of whether Maxforce was applied in the morning versus evening, in the spring, summer or winter, at lower or higher application rates, or in one versus multiple (up to four) applications. This is consistent with some laboratory trials that suggest that hydramethylnon is effective at killing Argentine ant workers but relatively ineffective at killing Argentine ant queens (Hooper-Bui and Rust 2000, 2001), although laboratory trials can be poor predictors of field efficacy (see below). Nevertheless, Maxforce remained an easy to use product with a relatively safe environmental profile (hydramethylnon breaks down quickly in sunlight) that yielded fairly good control, and therefore became both a useful standard against which to compare other bait products and a good bait with which to attempt a containment strategy in the park. This experimental containment strategy was implemented annually between 1997 and 2004, with attempts to improve results incorporated each year. It was finally discontinued after 2004, when it appeared that the size and/or quality of the Maxforce granule may have significantly reduced its effectiveness in both the perimeter containment plots and in smaller eradication experiment plots (see sections III.C.7 and III.D.2 above). Logistical difficulties inherent in the containment technique also contributed greatly to its termination. Currently, Maxforce Granular Insect Bait is being phased out and replaced with a different Bayer granular product named Maxforce Complete Granular Bait.

A second product, Maxforce FC Granular Bait, appeared to be more effective than the standard Maxforce Granular Ant Bait. This bait used the same granular bait carrier as Maxforce Granular Ant Bait, but differed in that it was formulated with fipronil instead of hydramethylnon. In at least one of our field plots, which was treated four consecutive times with Maxforce FC at one month intervals, very few nests survived (see section III.C.4 above). This result supports the contention that fipronil is a more effective toxicant than hydramethylnon (Hooper-Bui and Rust 2000, Stanley 2004). However, results were fairly inconsistent among plots treated with

Maxforce FC, and because this product is no longer manufactured, additional investigation of this bait is not possible.

The final ant bait that yielded fairly good results in our field trials was 0.5 HP Granular Ant Bait. This product, which was in development by Sumitomo Chemical Australia at the time of testing, held potential to be more effective than Maxforce Granular Ant Bait because it consisted of two different granular bait matrix types plus two different active ingredients. Like Maxforce, 0.5 HP contained hydramethylnon, but was also formulated with the insect growth regulator pyriproxyfen. This variety in both bait matrix and toxicant could overcome some of the variability in food preference and/or food selectivity that seems to be common in Argentine ant colonies. While both granule types of 0.5 HP appeared to be fairly attractive to ants at HALE, field tests of the bait produced results that were qualitatively very similar to those produced by Maxforce Granular Ant Bait: strong reduction in numbers of foraging workers, but substantial nest survival in the test plots.

Table 1. Ant baits screened for attractiveness and/or tested for efficacy at HALE, from 1994 to 2009. Baits are listed in approximate chronological order of testing during this period.

Name	**Toxicant**	**Formulation**	**Bait matrix**	**Type of test**[1]	**Plot size**	**Attractiveness/ Efficacy**[2]
[3]Maxforce Granular Ant Bait	hydramethylnon	granule	insect based granule	attractiveness, lab efficacy, field efficacy	25x25m 100x100m perimeter plots	attractive, poor in lab, good in field
Amdro granular ant bait	hydramethylnon	granule	corn grit/soy oil granule	attractiveness	n/a	not attractive
honey granules	none, blank	granule	honey based granule	attractiveness	n/a	moderately attractive
insect protein granules	none, blank	granule	insect based granule	attractiveness	n/a	not attractive
honey doughy bait	none, blank	doughy granule	honey based doughy granule	attractiveness	n/a	not attractive
high protein doughy bait	none, blank	doughy granule	insect based doughy granule	attractiveness	n/a	not attractive
fish protein bait	none, blank	doughy granule	fish based doughy granule	attractiveness	n/a	not attractive
25% sugar water	none, blank	liquid	sugar water	attractiveness	n/a	attractive
Grants Kills Ants	arsenic	solid, in bait station	unknown	attractiveness, field efficacy	25x25m	poor
Maxforce FC granular bait	fipronil	granule	insect based granule	field efficacy	100x100m	good
experimental sugar water bait	fipronil	liquid	sugar water	field efficacy	50x50m	poor
Pharorid Ant Growth Regulator	methoprene	user designed, used viscous liquid	tuna/corn syrup blend	field efficacy	100x100m	poor
Advance Granular Carpenter Ant Bait	abamectin	granule	corn grit/soy oil granule	attractiveness, field efficacy	100x100m	attractive, poor
Advance Granular Ant Bait	abamectin	granule	corn grit/soy oil granule	attractiveness	n/a	moderately attractive

CB-441 Granular Ant Bait with IGR	orthoboric acid, nylar	solid, in bait station	unknown	attractiveness	n/a	not attractive
Distance Fire Ant Bait	pyriproxyfen	granule	corn grit/soy oil granule	attractiveness	n/a	not attractive
protein paste	none, blank	paste	unknown protein	attractiveness	n/a	not attractive
Siege-Pro Fire Ant Bait	hydramethylnon	granule	corn grit/soy oil granule	attractiveness	n/a	not attractive
Tast-E-Bait	none, blank	granule	bread crumbs, other carbohydrates	attractiveness	n/a	not attractive
25% sugar water with 0.5% boric acid	boric acid	liquid	25% sugar water	attractiveness, field efficacy	50x50m	attractive, moderate
Pre-Empt Liquid Ant Bait	imidacloprid	liquid	sugar water	attractiveness	n/a	not attractive
Drax Ant Kil Gel	orthoboric acid	gel	sugar based gel	attractiveness	n/a	not attractive
Maxforce Carpenter Ant Bait Gel	fipronil	gel	unknown	attractiveness	n/a	not attractive
Maxforce FC Ant Killer Bait Gel	fipronil	gel	unknown	attractiveness	n/a	not attractive
Optigard Ant Gel Bait	thiamethoxam	gel	sugar based gel	attractiveness	n/a	not attractive
Gourmet Liquid Ant Bait	borate (DOT)	liquid	sugar water	attractiveness, field efficacy	120x140m	moderately attractive to attractive, poor
0.5 HP Granular Ant Bait	hydramethylnon + pyriproxyfen	granule	fish based granule + corn grit/soy granule	attractiveness, field efficacy	100x100m	attractive, good
Advion Ant Gel Bait	indoxacarb	gel	sugar based gel	attractiveness	n/a	not attractive to moderately attractive
Advion Ant Bait Arena	indoxacarb	solid, in bait station	unknown	attractiveness	n/a	not attractive to moderately attractive
Advion Insect Granule	indoxacarb	granule	unknown	attractiveness, lab efficacy, field efficacy	100x100m	moderately attractive, good in lab, poor in field

[1] Attractiveness tests include both formal bait preference tests as well as informal tests where the baits were offered to ants in the field to assess general attractiveness

[2] Attractiveness is grossly categorized as attractive, moderately attractive or not attractive; efficacy of field tests is indicated with the following rough categorizes: good (>75% reduction in foraging ants), moderate (>50% but <75% reduction in foraging ants), or poor (<50% reduction in foraging ants).

[3] Maxforce Granular Ant Bait was tested on multiple occasions, using different application rates, methods of application, concentrations of toxicant, and was tested both alone and in combination with other baits.

In summary, none of the commercially available baits that we tested was able to eradicate all nests in field plots, and therefore none was judged to work well enough to justify an eradication attempt at HALE. Maxforce FC came closest to this goal, however this bait was only marketed for a relatively short period. It is also notable that none of the liquid baits tested yielded good control, despite what appears to be a fairly strong preference for liquid carbohydrate foods by Argentine ants (Markin 1970, Rust et al. 2000). It is our belief that the failure of most of the commercial baits tested, whether granular, liquid, gel or paste, stemmed mainly from insufficient attractiveness of their bait matrices. Indeed, most of the ant baits on the market were largely ignored when placed in the field at HALE, including sweet liquids and gels that are normally very attractive to a large variety of ants. This is most likely due to the incorporation of preservatives and other additives that increase shelf life or ease of application (for example, see Appendix 6 for investigations that explored the effects of preservatives and other additives on the attractiveness of sugar water based baits).

Due to these problems with bait attractiveness, the Argentine Ant Project at HALE would probably need to explore the possibility of developing a specialized bait, rather than relying on a commercially produced bait, in order to achieve better results. This could involve modification of an existing bait, as was attempted with Gourmet Liquid Ant Bait, and would necessitate approval of and close cooperation with the manufacturer. An alternative would be to attempt to pursue approval to use Xstinguish bait, a commercial bait manufactured in New Zealand and not registered for use in the US. This paste bait was specially designed to be highly attractive to Argentine ants, has a shorter shelf life relative to most registered products, and therefore differs from most or all products available in the US. The bait has been used in what appears to be one of the few successful Argentine ant eradication efforts in the world: a roughly 13 ha infestation on Tiritiri Matangi Island near Auckland, NZ (C. Green pers. comm.). Both of these routes – development/modification of US registered baits or approval to use a foreign registered bait – would involve significant regulatory hurdles. Because the baits ultimately used would likely be liquid or paste in form, there would also be major logistical challenges in devising methods to successfully apply the baits across the two large ant populations at HALE.

In the meantime, the project has focused on a more immediate issue: the upper ant population has recently spread to the Holua campground and cabins. This raises the risk that this area will become a source pool for propagules carried to more distant parts of the park in the gear of campers or even park employees. We have been testing several liquid and gel baits, including Terro PCO liquid bait (borax active), Maxforce Quantum liquid bait (imidacloprid active), and Advion Ant Gel bait (indoxacarb active), in and around the cabins at this campsite, as well as around other structures in the park, including offices and greenhouses. The last of these baits, Advion Ant Gel, was previously found to be relatively unattractive at HALE, but possibly due to high resource demands resulting from very high densities of ants in the park during the summer and fall of 2010, this bait has been a useful tool in combination with the liquid baits offered in bait stations around the cabins and other structures. These bait stations, while somewhat successful on a small scale, would be difficult to use across a larger area.

A final lesson learned while testing ant baits at HALE is that results can be surprisingly different when baits are evaluated in different settings or at different scales. For example, some baits performed very well in bait preference tests, but produced little or no control when applied in field plots (e.g. Advance Granular Carpenter Ant Bait, Gourmet Liquid Ant Bait to a lesser degree), or did not perform as expected from the preference tests (e.g. different granule types in 0.5 HP Granular Ant Bait, see Appendix 7). In other cases, the effectiveness of baits was dramatically different when used on laboratory colonies in comparison to application in field plots (e.g. Maxforce Granular Ant Bait and Advion Insect Granules). This means that smaller scale testing may often be unreliable for accurately assessing the effectiveness of ant baits, and that the labor intensive use of fairly large field plots will continue to be necessary in the search for a more effective management strategy for Argentine ants in the park.

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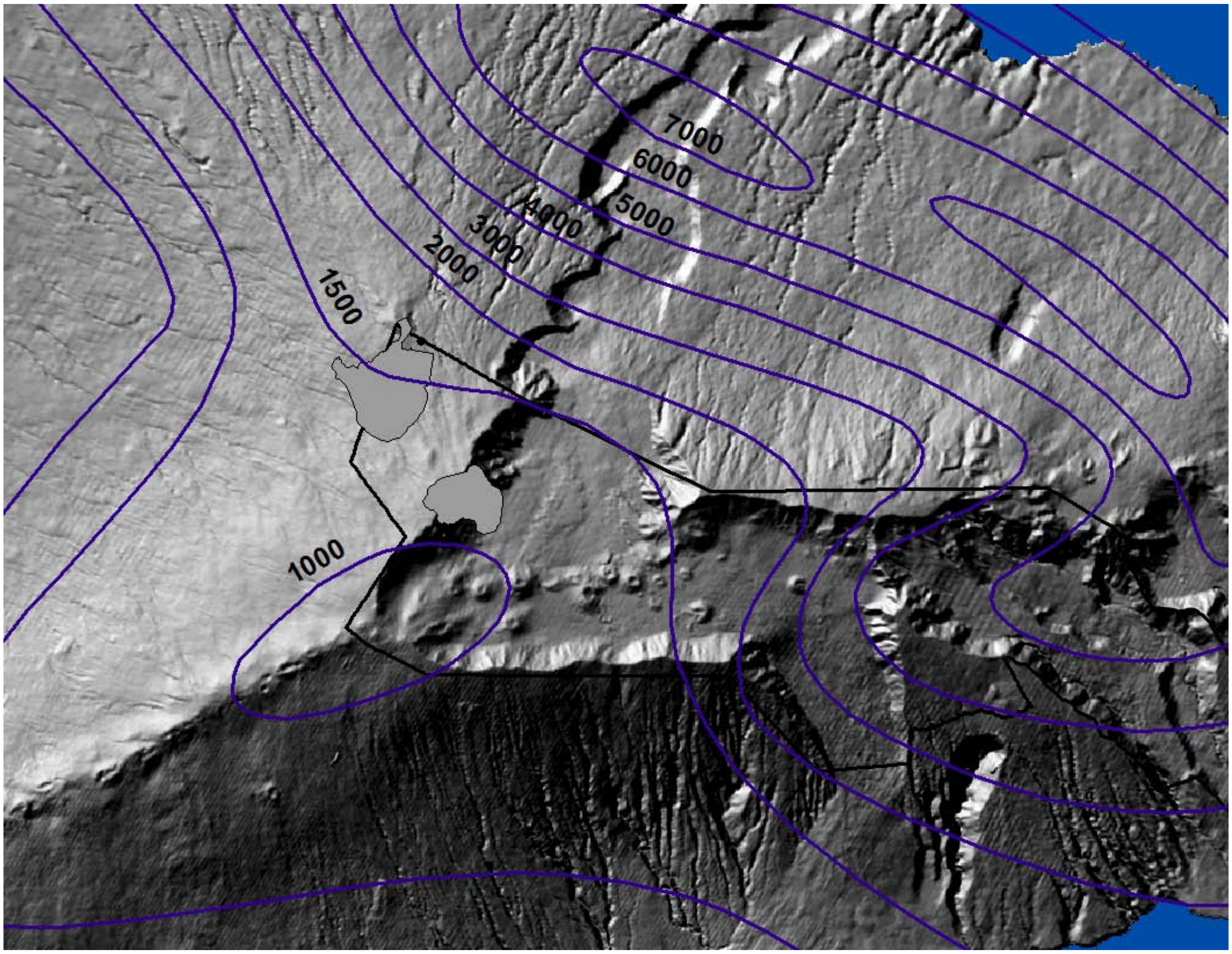
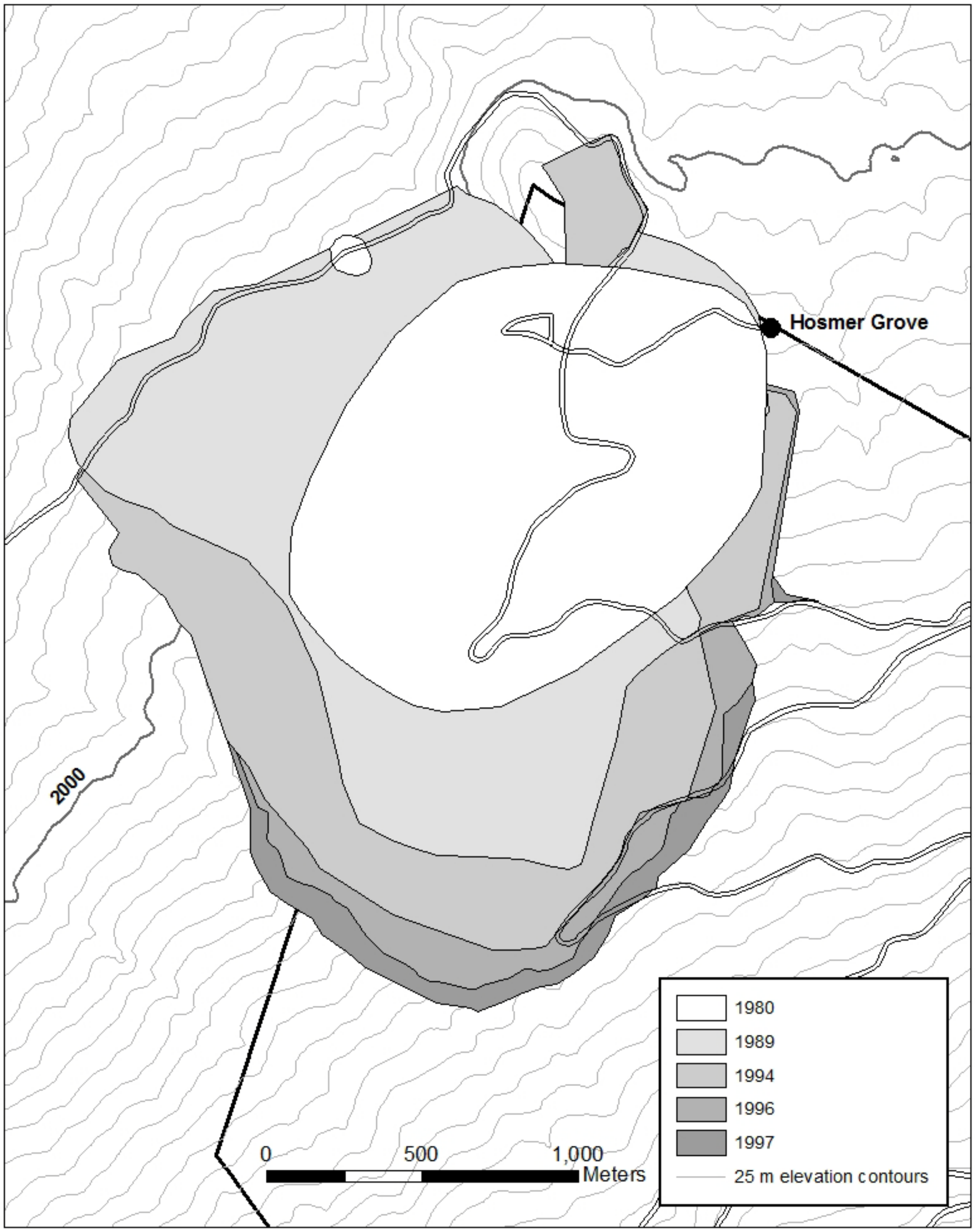
APPENDIX 1. Argentine ant patterns of spread at Haleakala National Park.

Figure 1. Locations of the lower and upper Argentine ant populations (1997 distributions shown in grey) in relation to the spatial rainfall pattern on the mountain. Median annual precipitation isohyets are shown in blue.



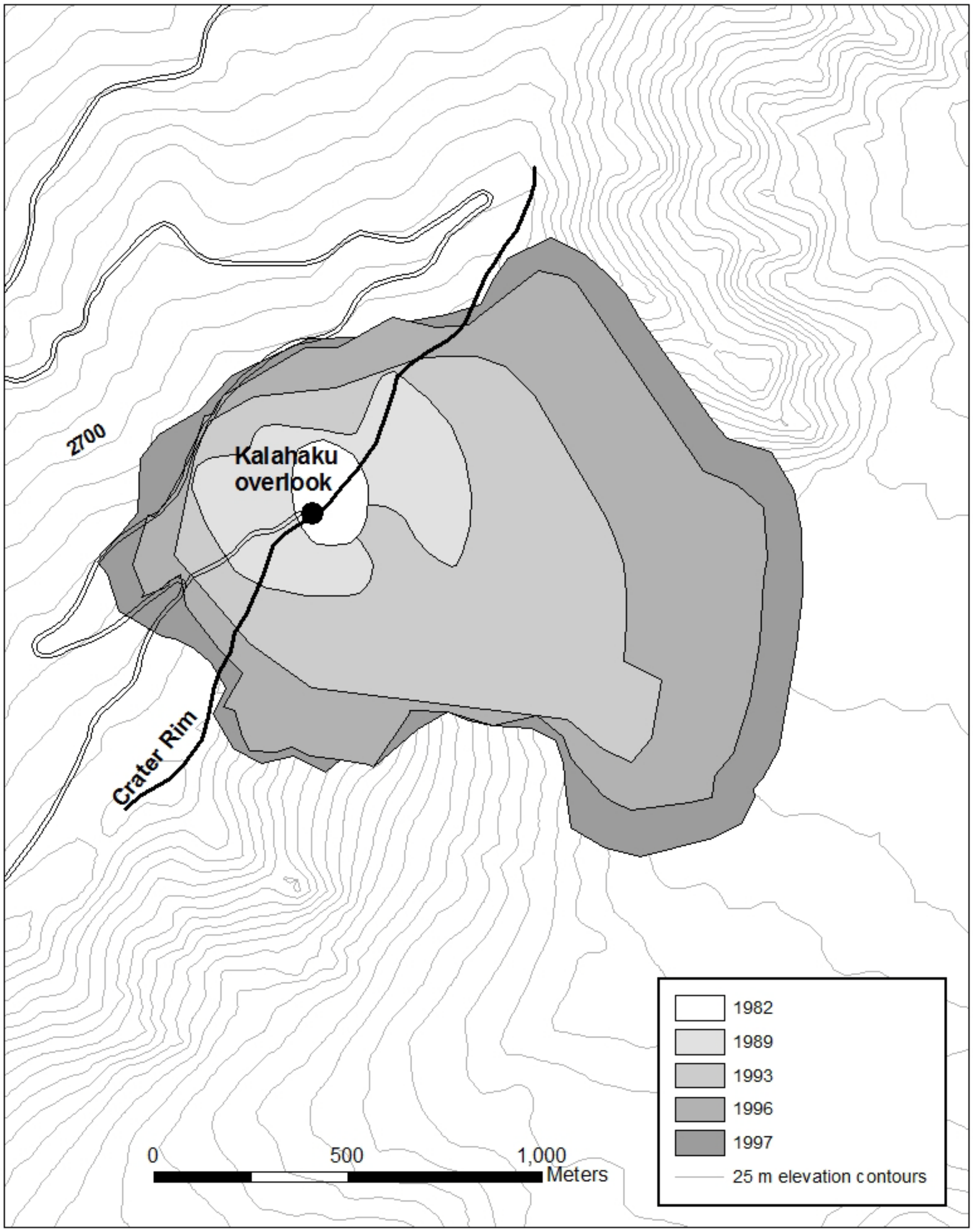
APPENDIX 1. Argentine ant patterns of spread at Haleakala National Park.

Figure 2. Pattern of spread in the lower Argentine ant population. Nearly all of the spread has been towards the southeast of Hosmer Grove, the site where ants were first recorded in the park. This corresponds to a leeward direction of spread.



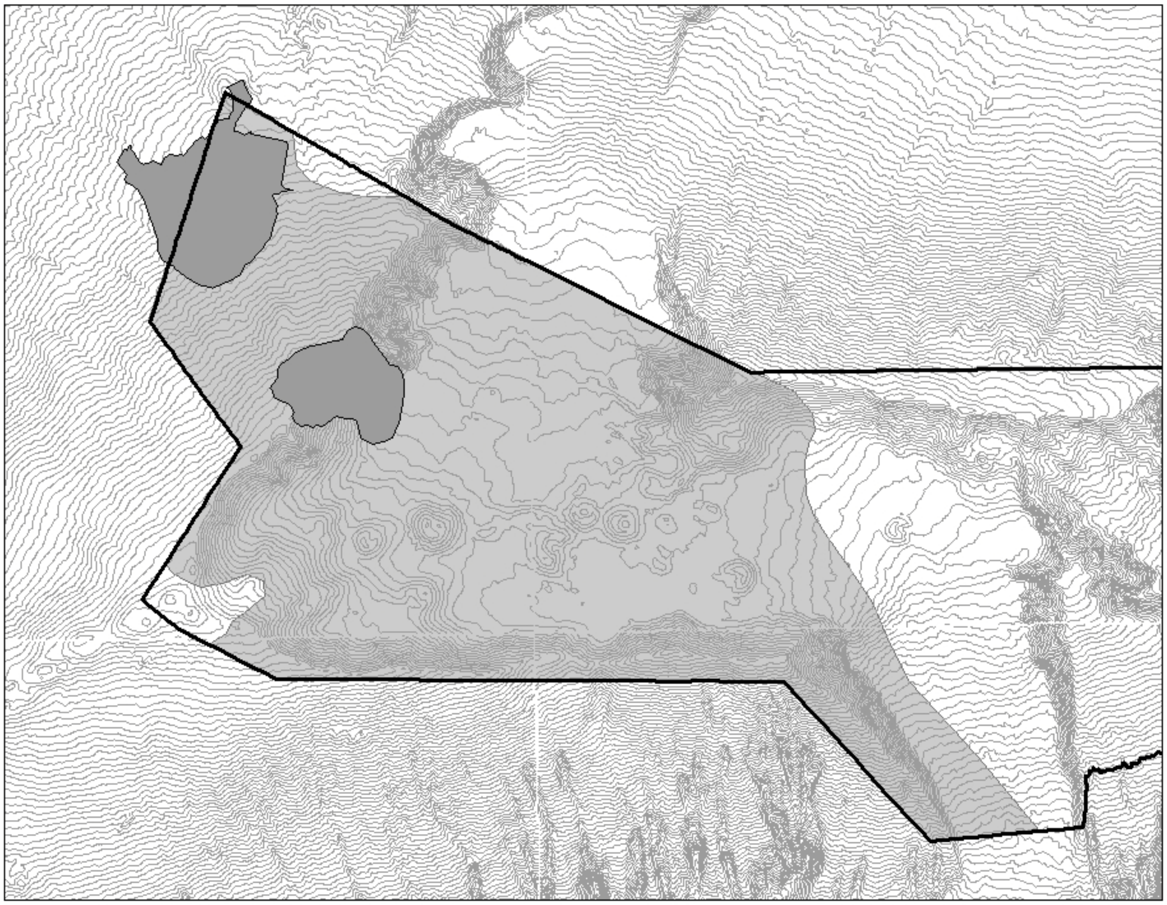
APPENDIX 1. Argentine ant patterns of spread at Haleakala National Park.

Figure 3. Pattern of spread in the upper Argentine ant population. The upper population has spread in all directions from its hypothesized point of origin at Kalahaku overlook, but has spread much faster towards the east and into the crater.



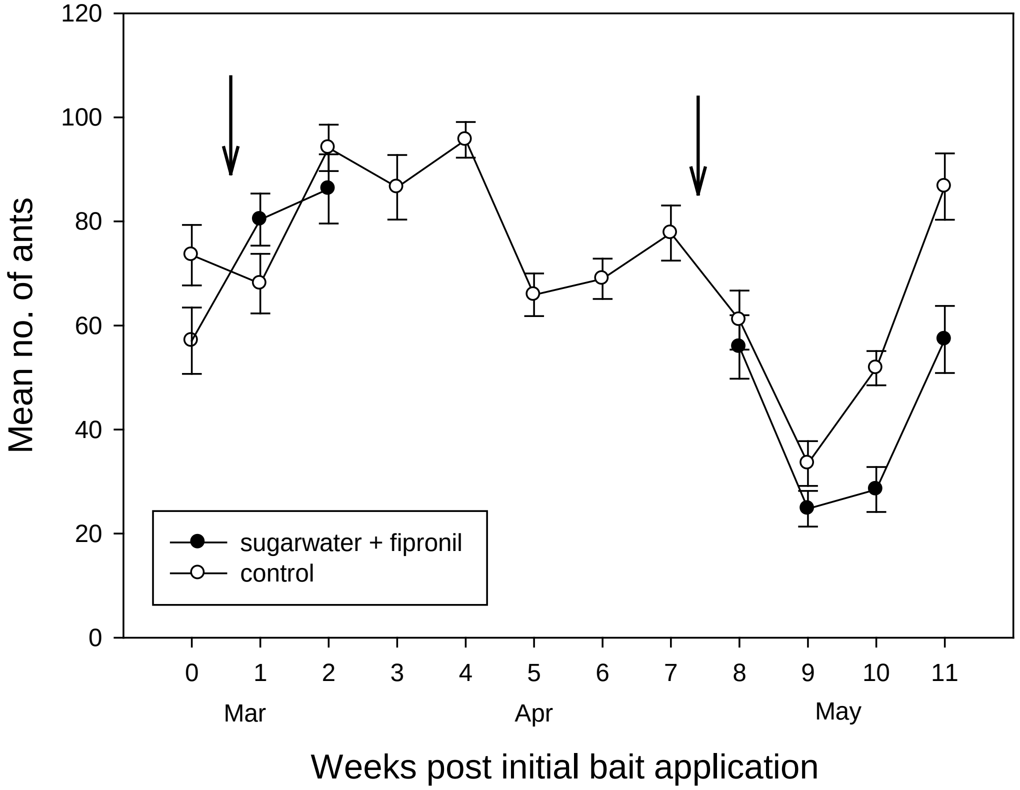
APPENDIX 1. Argentine ant patterns of spread at Haleakala National Park.

Figure 4. Rough prediction of the total potential range of the Argentine ant in HALE, made in 2004, based on known tolerances in rainfall and temperature (using elevation as a proxy), as well as judgments about microhabitat requirements. Potential range is shown in light grey, and ant population distributions as of 1997 are shown in darker grey. The thick black line indicates the park boundary in 2004.



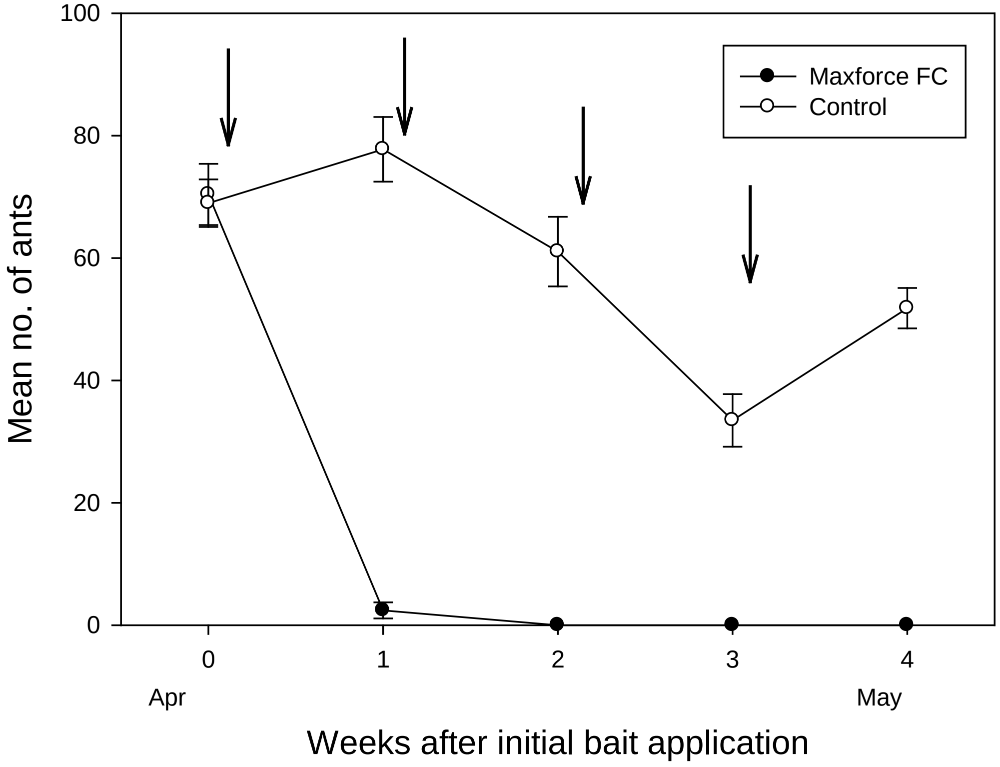
APPENDIX 2. Results from the 1998 eradication plots.

Figure 1. Mean numbers of ants (±1 SE) at monitoring bait cards (n=29) in the plot that received the sugar water/fipronil bait, compared to the no treatment control plot. Arrows indicate when the two different formulations of the sugar water/fipronil bait were placed in the field. Because of apparent repellency of the first formulation, baits were removed from the plot in March and replaced with the second formulation on 4/24/98.



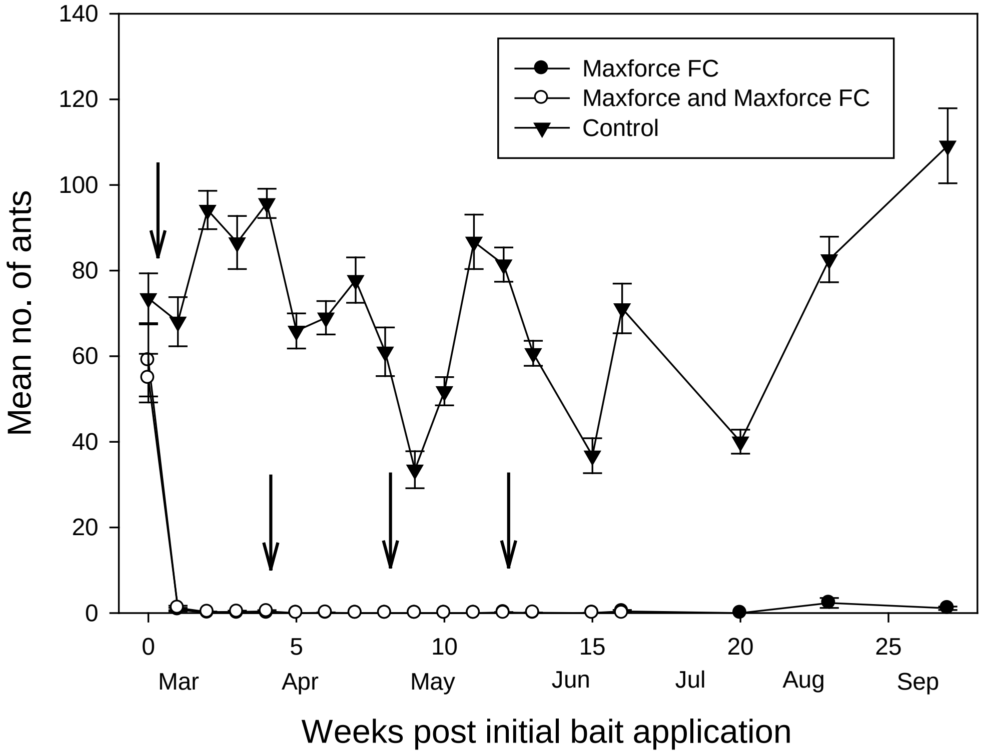
APPENDIX 2. Results from the 1998 eradication plots.

Figure 2. Mean numbers of ants (±1 SE) at monitoring bait cards (n=29) in the plot that received four consecutive applications, each separated by one week, of the Maxforce FC granular bait, compared to the no treatment control plot. Arrows indicate the timing of the four broadcast applications.



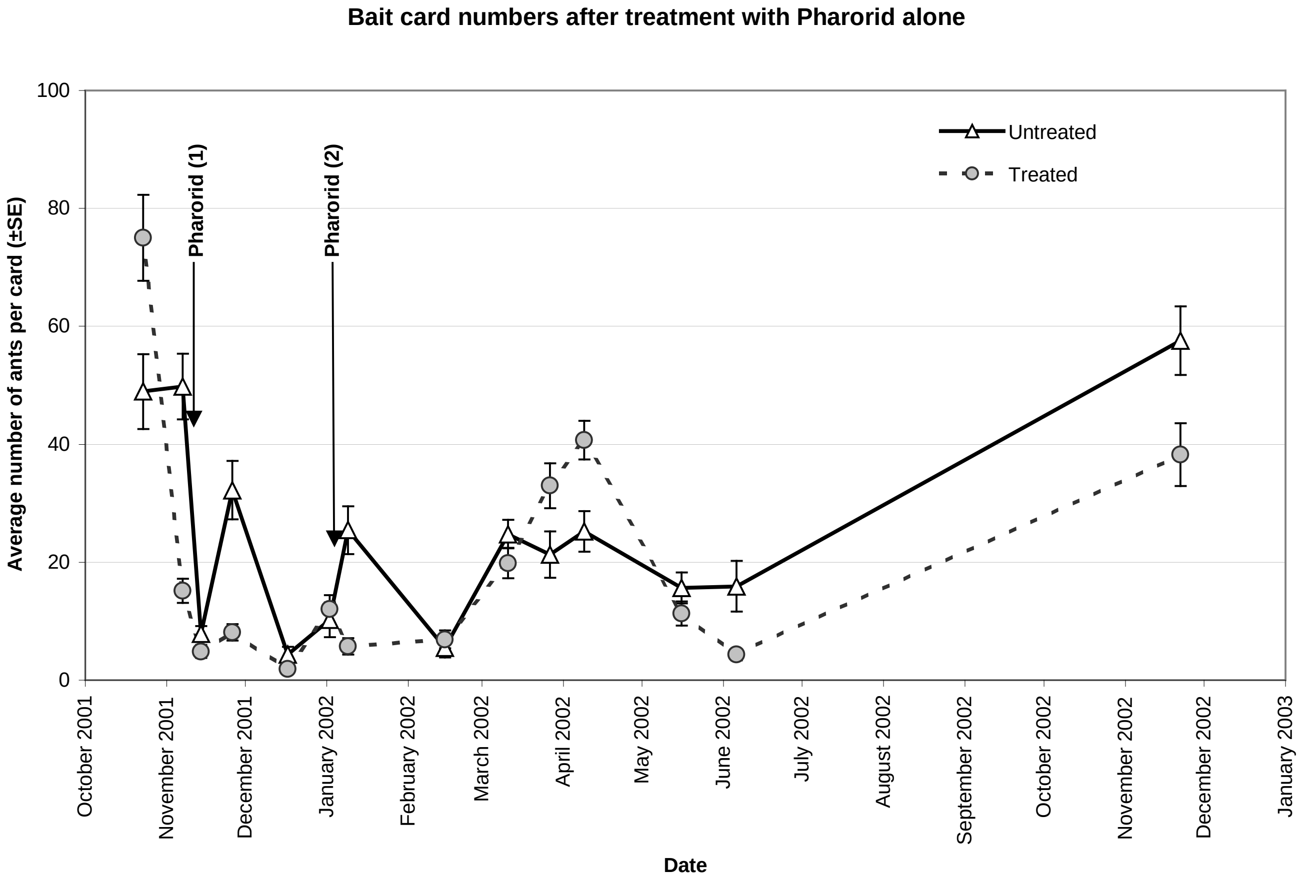
APPENDIX 2. Results from the 1998 eradication plots.

Figure 3. Mean numbers of ants (±1 SE) at monitoring bait cards (n=29) in the two plots that received four consecutive bait applications, each separated by one month, compared to the no treatment control plot. One plot received one application of standard Maxforce Granular Ant Bait, followed by three applications of Maxforce FC granular bait, while the second plot received four applications of Maxforce FC. Arrows indicate the timing of the four broadcast applications.



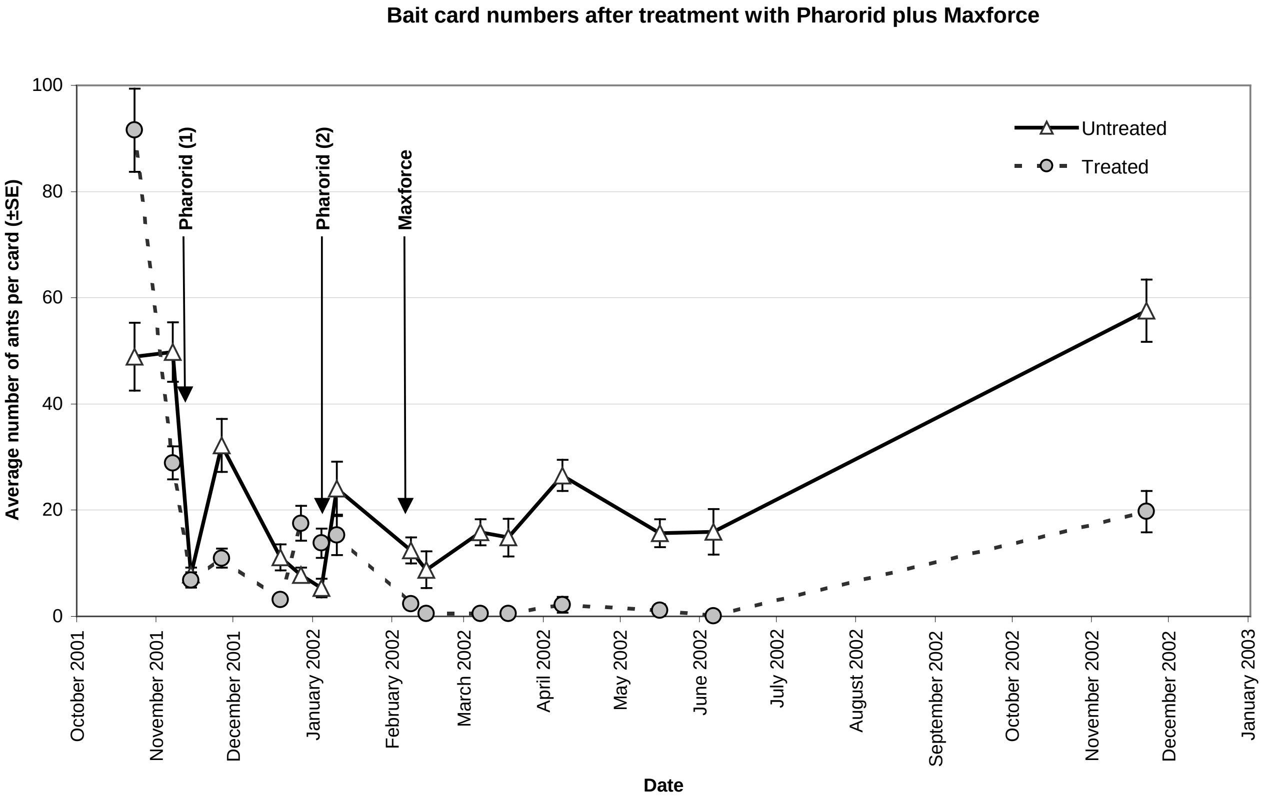
APPENDIX 3. Results from the 2001-2002 Pharorid and Maxforce eradication plots.

Figure 1. Number of foraging workers at bait card monitoring stations in the plot treated with Pharorid, compared to the untreated control plot. Arrows indicate the two dates when Pharorid was offered in bait stations in the plot.



APPENDIX 3. Results from the 2001-2002 Pharorid and Maxforce eradication plots.

Figure 2. Number of foraging workers at bait card monitoring stations in the plot treated with Pharorid followed by Maxforce, compared to the untreated control plot. Arrows indicate the two dates when Pharorid was offered in bait stations in the plot, and the date when Maxforce Granular Insect Bait was broadcast in the plot.



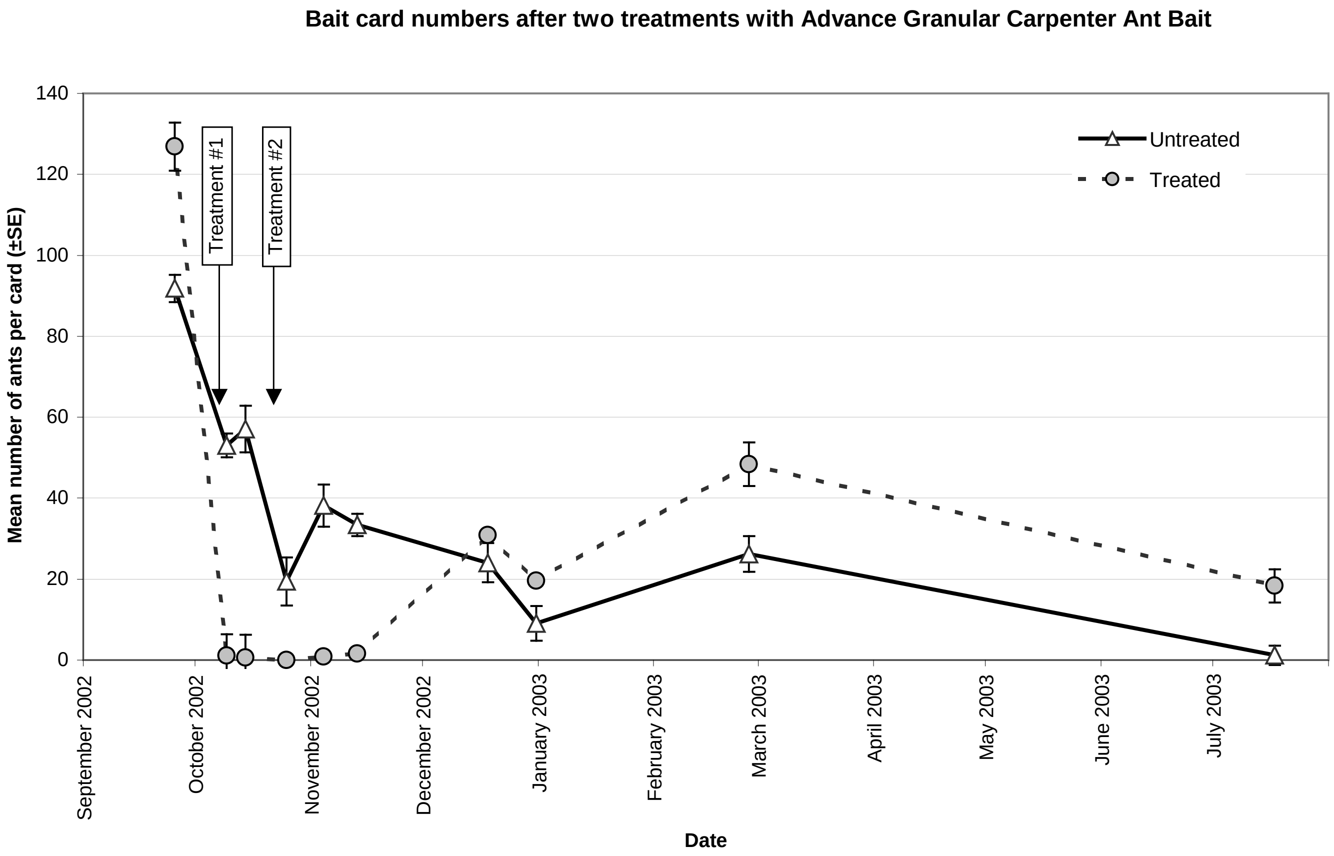
APPENDIX 4. Results from the 2002-2003 bait acceptance trials and eradication plots.

Table 1. Bait acceptance by the Argentine ant at HALE. The amount of bait taken was estimated by subtracting the mean weight change of control baits from the mean weight change of offered baits, and hence can be slightly negative (indicating no real bait uptake). Note the attractiveness of Advance GCAB compared to Maxforce GIB, the bait most often used at HALE.

Product name	**Manufacturer**	**Bait ingredients**	**Active ingredients**	**Amount of bait taken (g)**
Advance Granular Ant Bait	Whitmire Micro-Gen Inc.	corn grit, soybean oil	abamectin	0.059
Advance Granular Carpenter Ant Bait	Whitmire Micro-Gen Inc.	corn grit, soybean oil	abamectin	0.665
CB-441 Granular Ant Bait with IGR	Waterbury Companies Inc.	????	orthoboric acid, nylar	-0.005
Distance Fire Ant Bait	Valent USA Corp.	corn grit, soybean oil	pyriproxyphen	-0.002
Maxforce Granular Insect Bait	Clorox Corp.	ground silkworm pupae	hydramethylnon	0.008
Protein Paste	????	unknown protein	none	0.002
Seige Pro Fire Ant Bait	Waterbury Companies Inc.	corn grit, soybean oil	hydramethylnon	-0.003
Tast-E-Bait	????	bread crumbs, other ingreds.	none	-0.008

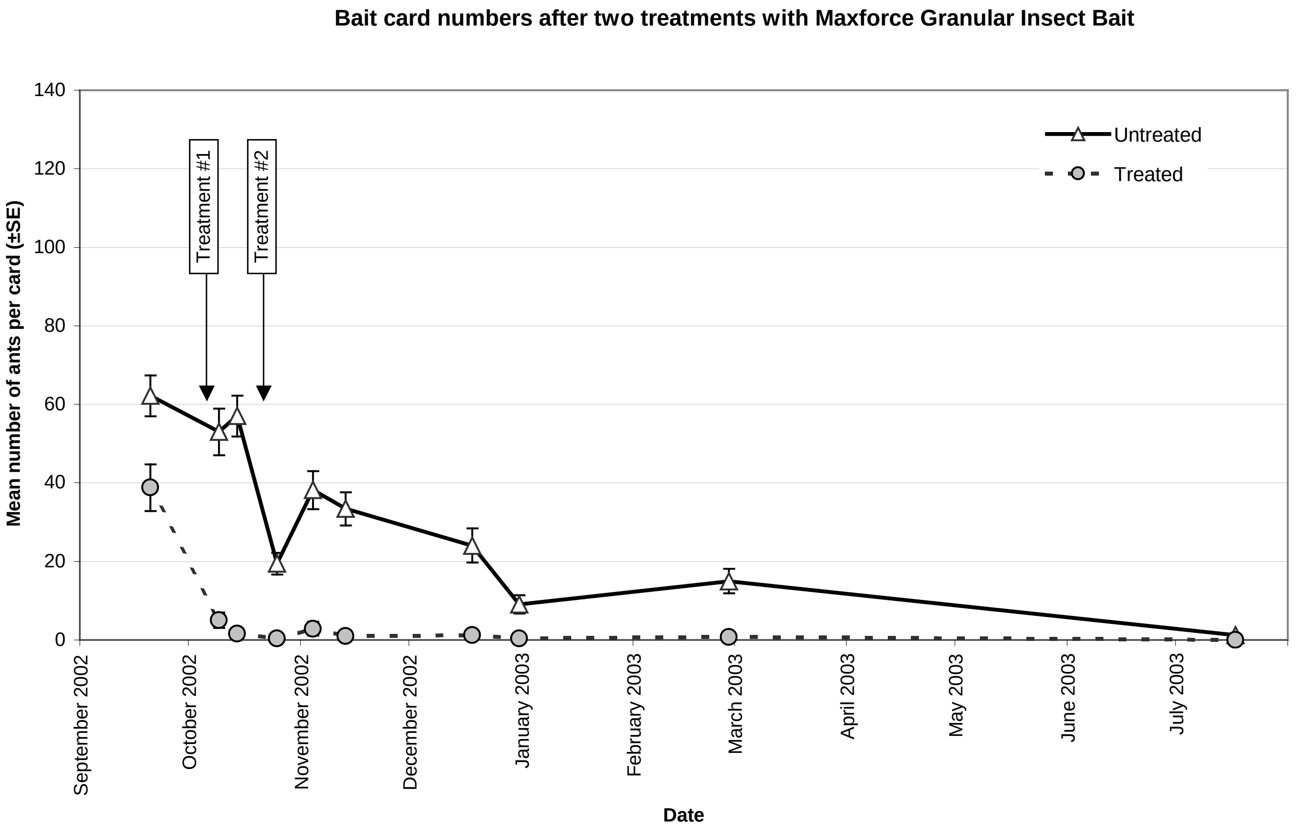
APPENDIX 4. Results from the 2002-2003 bait acceptance trials and eradication plots.

Figure 1. Number of foraging workers at bait card monitoring stations in the plot treated twice with Advance CGAB, compared to the untreated control plot.



APPENDIX 4. Results from the 2002-2003 bait acceptance trials and eradication plots.

Figure 2. Number of foraging workers at bait card monitoring stations in the plot treated twice with Maxforce GIB, compared to the untreated control plot.



APPENDIX 5. Results from the 2004-2005 eradication plots.

Figure 1. Mean number of ants at bait card monitoring stations in each plot. The arrows represent each of the four Maxforce broadcast applications. Boric acid in sugar water was available continuously.

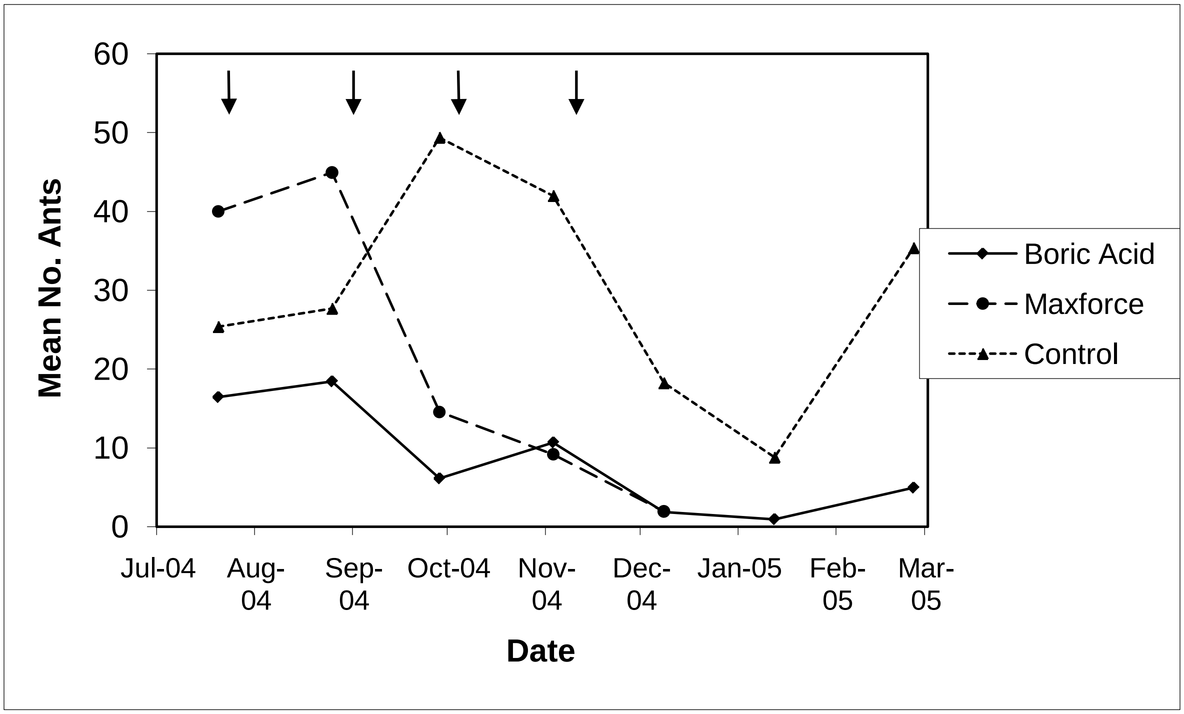
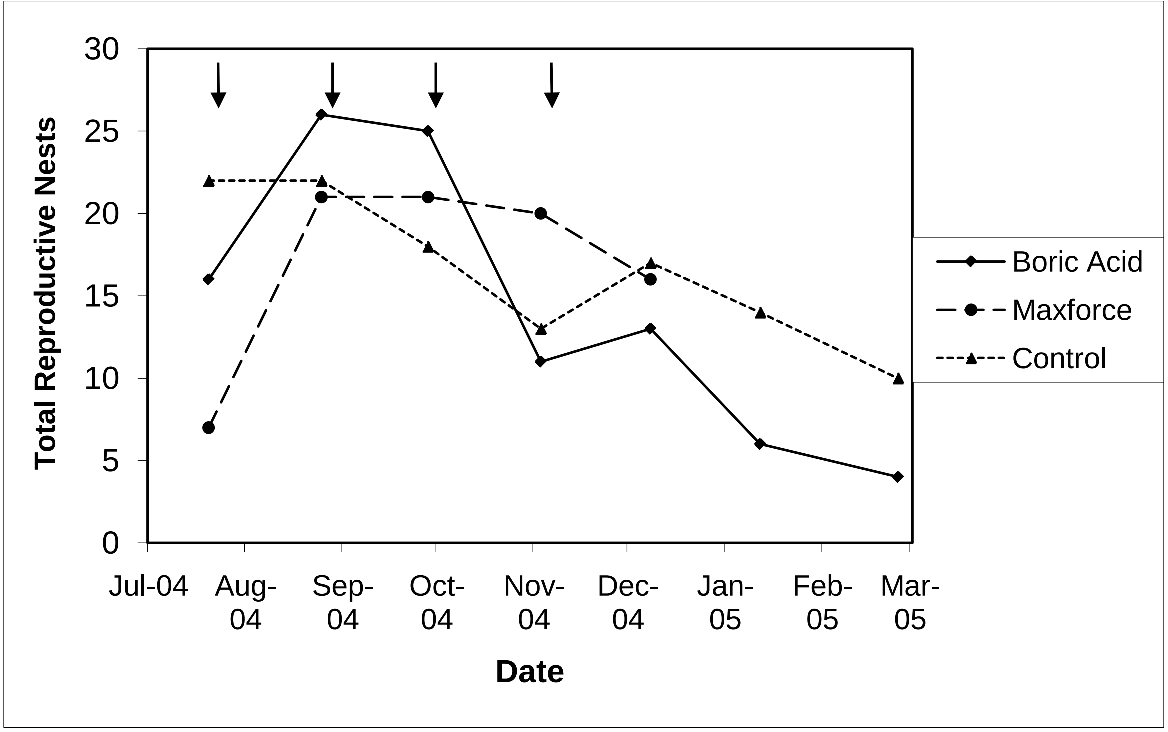


Figure 2. Total number of reproductive nests per quadrat in each plot. The arrows represent each of the four Maxforce broadcast applications. Boric acid in sugar water was available continuously.



APPENDIX 5. Results from the 2004-2005 eradication plots.

Figure 3. Average worker larvae abundance class per nest in each plot. Abundance classes are 0 = 0, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 101-500, 5 = >500. The arrows represent each of the four Maxforce broadcast applications. Boric acid in sugar water was available continuously.

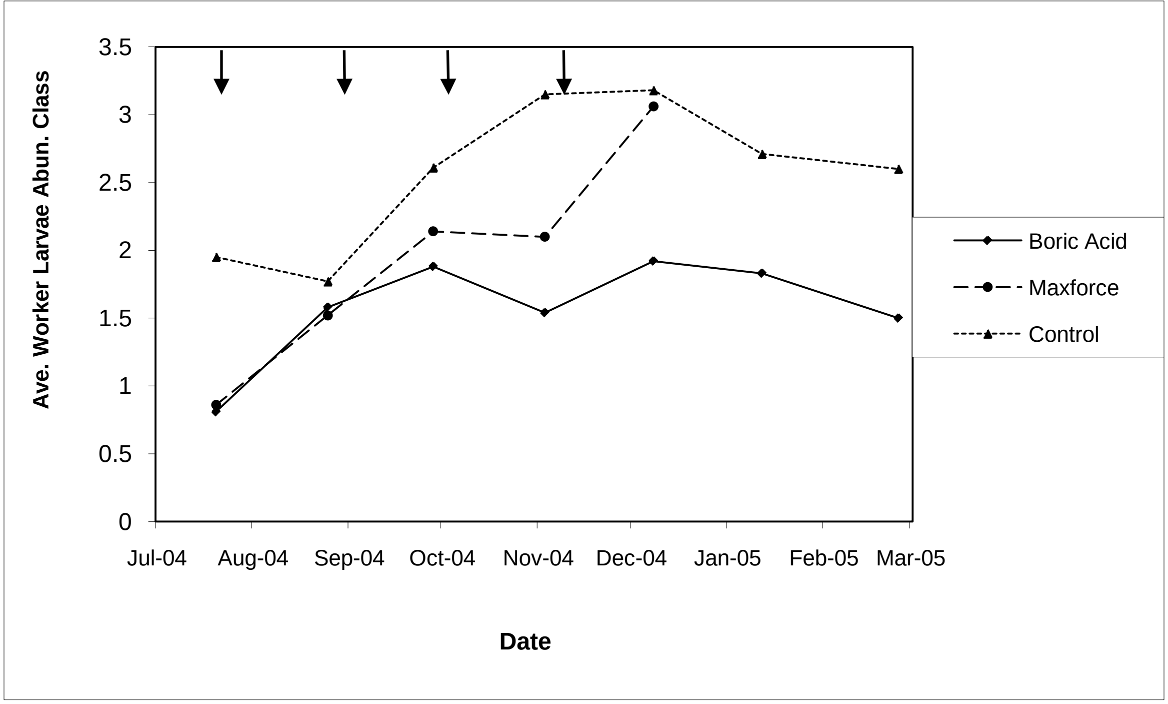
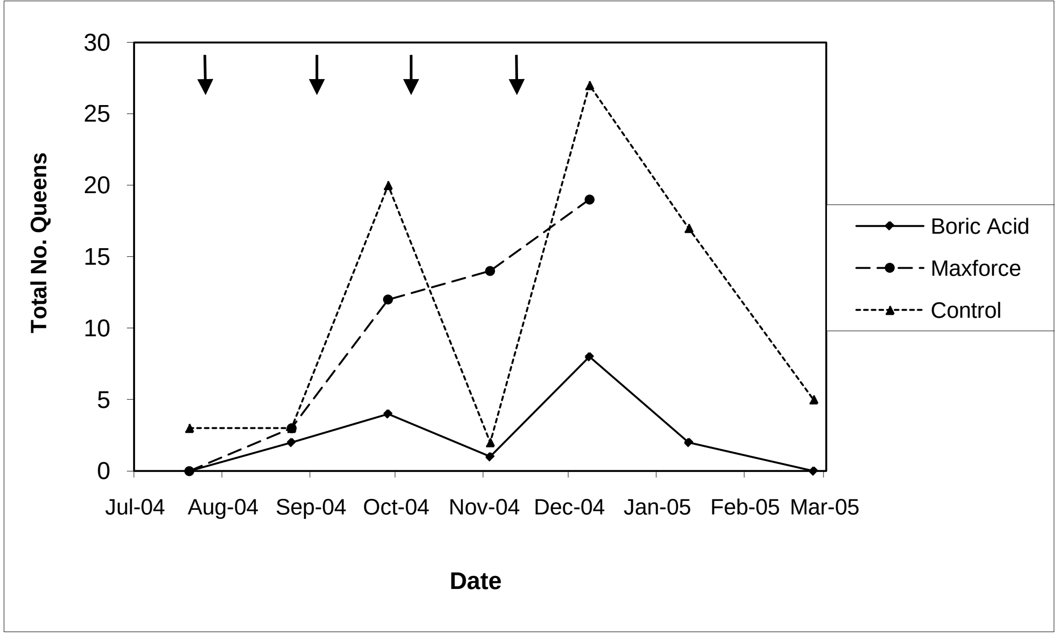
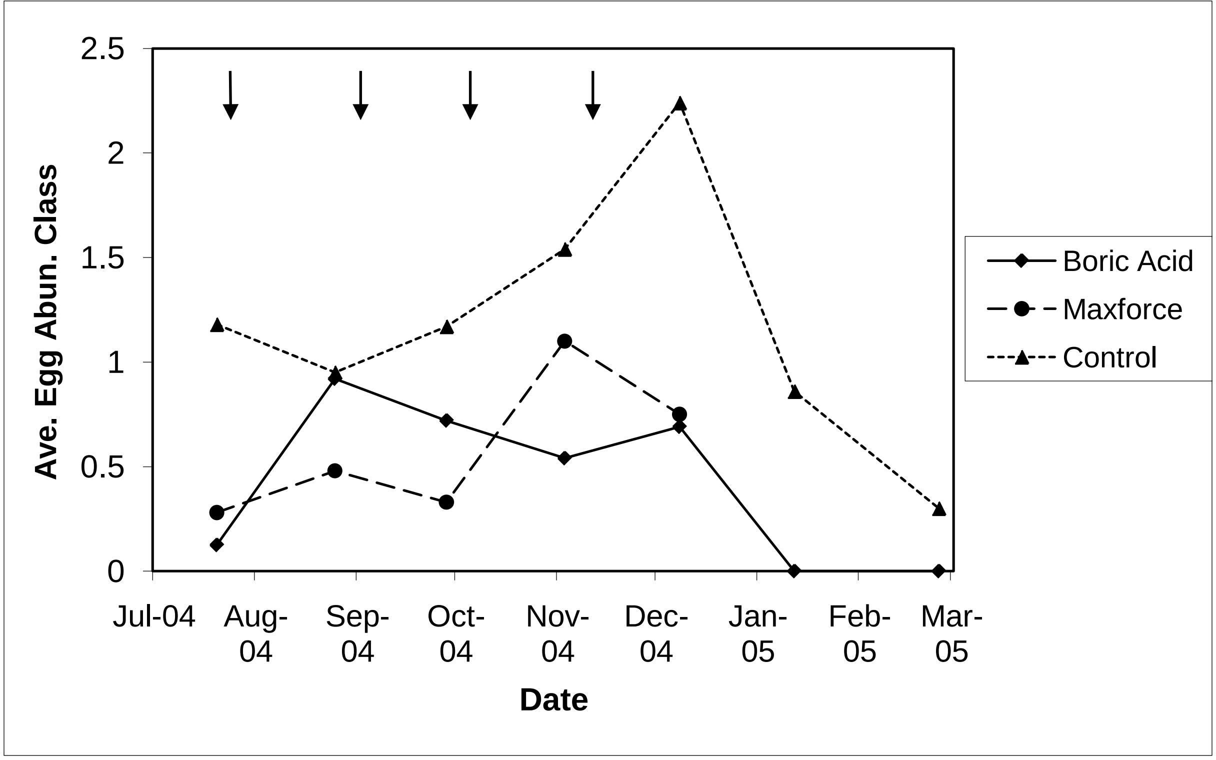


Figure 4. Total number of queens per quadrat in each plot. The arrows represent each of the four Maxforce broadcast applications. Boric acid in sugar water was available continuously.



APPENDIX 5. Results from the 2004-2005 eradication plots.

Figure 5. Average egg abundance class per nest in each plot. Abundance classes are 0 = 0, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 101-500, 5 = >500. The arrows represent each of the four Maxforce broadcast applications. Boric acid in sugar water was available continuously.



APPENDIX 6. A full report on the evaluation of Gourmet Liquid Ant Bait is provided in the following pages.

DEVELOPING TECHNIQUES FOR INVASIVE ANT CONTROL USING LIQUID BAITS: A TEST OF GOURMET LIQUID ANT BAIT ON ARGENTINE ANTS AT HALEAKALA NATIONAL PARK

October 2008

Report to Hawaii Invasive Species Council for PCSU contract 438221

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EXECUTIVE SUMMARY

I investigated the potential of Gourmet Liquid Ant Bait, which contains a borate active ingredient, as a tool for eradicating Argentine ants in Haleakala National Park (HALE), Hawaii. I discuss the advantages and difficulties of using liquid baits for Argentine ant control at this site, as well for invasive ant control in Hawaii in general. Three main topics were addressed: selection of bait station design, modification of bait formulation to improve attractiveness and consumption, and a test of the efficacy of Gourmet bait against Argentine ants in experimental plots in the park.

After evaluation of an alternate commercial bait station, Km AntPro, I decided that a home-made bait station design was better suited for the situation at HALE. This bait station consisted of an outer case made from pvc tubing and end caps, and an inner tube that housed the liquid bait. Although the commercial bait station was cheaper per unit volume of bait, the home-made pvc station was much cheaper per bait station, which was deemed preferable for HALE where more bait stations each holding less bait is likely to be most effective. Moreover, the home-made station was easier to use in uneven terrain, less subject to evaporative water loss, and better at excluding non-target arthropods.

There was a surprising amount of difficulty with attractiveness of the Gourmet bait. I investigated a number of modifications to the bait formulation in an attempt to improve attractiveness, including addition of protein (in the form of hydrolyzed casein), dilution with water and sugar water, adjustment of pH with sodium bicarbonate, and finally dilution with different fruit juices. Favorable results in bait preference tests, however, did not translate into high levels of attractiveness or consumption in the experimental ant control plots. This was true for two different formulations that were tested in the plots consecutively (in an effort to improve bait performance): 75% Gourmet + 5% casein, diluted with 25% sugar water, initially, and 50% Gourmet + 5% casein + 45% grape juice, subsequently.

Because of the low attractiveness of the bait formulations, there was a low level of ant control over the course of 32 weeks in the two experimental treatment plots that were situated on an ant population boundary at HALE. The two plots tested two different bait application rates: stations placed in either a 10 m grid pattern or a 20 m grid pattern within the plots. Foraging ant numbers in both treatment plots appeared to be impacted only slightly, and eradication was clearly not achieved: reproductive nests, which possessed queens, eggs and worker larvae, persisted throughout the experiment. Finally, the Gourmet treatments did not prevent outward spread of the ant population boundary. Ants spread furthest from the control (no treatment) plot (mean of 39.6 m from May to December 2007), but not significantly more so than from the 10 m grid treatment plot (mean of 29.7 m).

Despite the poor results in this study, I believe that sugar water based liquid baits should be tested in other locations and with other invasive ant species because of the well known importance and attractiveness of liquid carbohydrate food sources for invasive ants in general. Feeding preferences of ants can be variable both spatially and temporally, and liquid baits may prove to be an important control tool for certain use patterns where other baits are prohibited.

1. INTRODUCTION

Invasive ants are among the most damaging of Hawaii's invasive species. There are believed to be no native ants in Hawaii, yet in the past several hundred years over 50 ant species have been introduced to the state. Some of these species have caused substantial impacts to native Hawaiian biodiversity, and are pests of agriculture and urban areas (Krushelnycky et al. 2005). In addition, recent and potential introductions, such as the little fire ant and red imported fire ant, respectively, have the ability to exert strong impacts on tourism and other sectors of the economy (Gutrich et al. 2007).

Techniques for controlling and even eradicating existing populations of the state's most invasive ant species are critical for rapid response to incipient incursions, as well as situations in which biodiversity and other interests can be protected by removing well-established but localized ant populations. Some of the most damaging invasive ant species exhibit a unicolonial social structure in which mating flights do not occur, new queens bud from existing nests and disperse short distances by walking, and populations can therefore exist as discrete, localized entities even when the species' total distribution is much wider. Successful eradication of local populations can thus result in the permanent removal of these species from particular areas of concern, as long as re-introduction by humans can be prevented or quickly detected (Krushelnycky et al. 2005, Silverman and Brightwell 2008).

Efforts to control or eradicate invasive ant populations typically involve the use of attractive baits formulated with insecticidal toxicants. However, different ant species respond to different baits, and different situations call for different active ingredients and methods of application. Developing multiple management tools for invasive ants will greatly improve the state's ability to address these problem species.

Most ant baits used for outdoor applications come in a granular form, making them relatively cheap and easy to disperse across the target area. Liquid baits are more difficult to apply, usually requiring some type of bait station to house the bait, and have therefore typically been used within homes or other structures where a relatively small number of stations are needed. This makes liquid baits more laborious and costly than granular baits, but liquid baits can have certain advantages over granular products. First, most liquid baits use sugar water as their base, which is highly attractive to a wide variety of invasive and pest ant species (Klotz and Moss 1996, Klotz et al. 1996, Klotz et al. 1997a, Klotz et al. 1997b, Klotz et al. 1998, Ulloa-Chacon and Jaramillo 2003). While some granular products work well for some ant species, other invasive ant species such as the Argentine ant (*Linepithema humile*) have so far proved to be insufficiently responsive to currently available commercial granular baits. Second, because they are ingested and transported internally, liquid baits are easily retrieved and shared among nestmates through trophallaxis. Third, liquid baits in bait stations can be monitored and replenished to provide longer and more thorough access for the target ants (while limiting access for non-target species). Finally, a number of commercial liquid baits are formulated with borate toxicants, which tend to be regarded as relatively benign pesticides (e.g., see below). Boric acid has been used to suppress ants in homes for decades (Klotz et al. 1997b), and recent laboratory and field studies suggest that when formulated at low concentrations (0.5-1%) in sugar water baits, boric acid can be as effective or more effective against certain ants than more recently developed toxicants (e.g. Hooper-Bui and Rust 2000). For all of these reasons, there has been renewed interest in liquid baits formulated with boric acid or other borates for use in certain agricultural and urban settings (Klotz et al. 1998, Daane et al. 2006, Greenberg et al. 2006, Nelson and Daane 2007), and these same features also make these baits an attractive potential option for ant control in sensitive natural area situations.

At Haleakala National Park (HALE), the Argentine ant has emerged as one of the most important threats to endemic subalpine shrubland and alpine zone arthropods. Since at least 1967, the Argentine ant has been slowly but steadily spreading within the park, with two discrete populations now covering over 625 ha. Numerous experiments testing a variety of commercial and experimental pesticidal ant baits have been conducted over the past ten years at HALE in an attempt to develop a method for eradicating the Argentine ant (e.g. Krushelnycky and Reimer 1998a,b). While some of these baits have been very effective in reducing numbers of ants, none has been able to eliminate all nests in experimental plots. Consequently, no ant bait product tested to date appears to be effective enough to successfully eradicate the two Argentine ant populations in the park. Continued research with additional products is therefore needed to address resource management goals specific to HALE, but also to improve capacity to manage invasive ants in Hawaii in general. In the present study, I evaluate whether Gourmet Liquid Ant Bait has the potential to eradicate Argentine ants at HALE. The methods developed and results obtained should also have direct relevance to other situations across the state.

Gourmet Liquid Ant Bait, manufactured by Innovative Pest Control Products, uses a mixture of several different sugars as attractants and the borate DOT (disodium octoborate tetrahydrate) for the active ingredient (at 1% concentration). I chose to test this bait among commercially available sugar water based baits for several reasons. First, Gourmet was previously found to be both attractive to and effective against Argentine ants in California (Greenberg et al. 2006). Second, the Gourmet label includes language permitting the user to dilute the bait with any food grade products that might increase bait attractiveness. The borate concentration in the bait can also be decreased in this way. Finally, Gourmet Liquid Ant Bait is registered for agricultural use (in approved bait stations), and Innovative Pest Control Products has obtained a Special Local Need label amendment allowing Gourmet to be used in organic fruit and nut orchards in California. The flexibility afforded by these types of provisions could be very important in Hawaii, for example in situations where new ant incursions occur on agricultural lands. Importantly, the owner of Innovative Pest Control Products expressed interest in working towards registration of wider use patterns in Hawaii should results be favorable.

This report covers three main areas of research relevant to testing Gourmet Liquid Ant Bait at HALE: the selection of an appropriate bait station design; work aimed at improving attractiveness and consumption of bait formulations; and testing the efficacy of the bait for eradicating Argentine ants in experimental plots.

2. BAIT STATION SELECTION

Delivery of liquid bait presents several unique challenges. First, the bait must be contained in a bait station that prevents, or at least greatly minimizes, spilling. Second, the bait station must minimize evaporation of the liquid bait because evaporation causes an increase in the concentration of the active ingredient. This is particularly important for baits containing very slow-acting toxicants, like borates, that must be offered for relatively long periods of time. Third, in many situations bait stations should be designed so as to minimize access to non-target species, such as pollinators that may be attracted to nectar-like sugar water based baits. This issue is again important for baits that are available for longer time periods. Finally, the bait station design should reflect some consideration of the practical difficulties of deploying liquid baits, such as ease of refilling, as well as cost of the station.

There are not many commercially produced bait stations that are designed to hold liquid baits and adequately address the conditions listed above. Among these, the Km AntPro bait station is well-designed for orchards and other agricultural settings (e.g. Greenberg et al. 2006), and comes highly recommended by some of its users. I evaluated this bait station for use at HALE, and decided that it would be unsuitable due to the following drawbacks. 1) Its size and bright green color would make it very conspicuous in open habitats in the park, such as around the crater rim, which would likely become an aesthetic issue for the park. 2) It needs to be placed such that it is nearly level, otherwise the bait spills and will continue to drain from the station. This is a particular challenge on the side of a mountain. 3) To work properly, the station should be filled with at least 10 oz of liquid bait. While such a large quantity per station is appropriate for orchards or other areas with extremely high densities of pest ants, it is likely too much for the situation at HALE, and probably some other natural areas, where the deployment of more stations containing less bait is likely to be more effective. 4) The design allows easy access to all relatively small arthropods, which includes most species at HALE. Of particular concern would be continuous access to the bait by native *Hylaeus* bees. 5) One station was found to be leaking one warm afternoon, with considerable amounts of the bait pouring out. This may have been caused by high temperatures inside the station leading to expansion and subsequent forcing of the bait through the “stress ducts” at the bottom. This seemed to set up an extended bout of leaking, possibly due to surface tension drawing out the bait. 6) Finally, one study (Klotz et al. 2004) found that the station has a fairly high level of evaporation, leading to an estimated 13-fold increase in concentration of active ingredient in at least one scenario. This is probably due to the design in which the accessible bait sits exposed in a feeding trough, where evaporative surface area is fairly large. While the above reasons make the Km AntPro bait station inappropriate for use at HALE and in other similar situations, it may still be an effective station in other settings such as orchards or nurseries.

No other commercially available bait station appeared likely to be suitable for HALE. I instead returned to a home-made design previously used at HALE to deliver liquid baits to Argentine ants. This design uses pvc pipe with solid endcaps as an external housing and a smaller internal tube for bait delivery. The small holes drilled in the external pvc endcaps allow access for ants but physically exclude all but the smallest non-target arthropods (probably including *Hylaeus*), and previously no organisms other than slugs were found inside them (and these very rarely). For this study, I considered a modification to the design of the internal delivery system, which originally consisted of a large cotton ball plugging the liquid bait in an open 50 ml plastic centrifuge tube. An alternative design could use Weedblock perforated nursery material as a membrane, secured with the centrifuge tube cap (with a hole drilled in it for access), to retain the liquid (Greenberg et al. 2006). This system might have lower rates of evaporation and may allow easier access to the bait for ants.

I tested three bait stations with the Weedblock design against three stations using the cotton ball design. I found that Weedblock only retains liquid when the tube is oriented vertically, i.e. when no air can relieve the vacuum pressure in the top of the tube. I therefore had to mount the Weedblock stations with rubber bands to wooden stakes. In contrast, the cotton ball stations were placed on the ground, such that the stations were positioned with at least a slight incline, and the open end of the internal centrifuge tubes were thus slightly raised to avoid spillage. Both stations employed external pvc outer tubes with four 5/64 inch holes drilled in each endcap. Because the weedblock design required the use of the centrifuge tube cap to secure the weedblock membrane, 1.5 inch diameter pvc pipe and endcaps were needed to accommodate this slightly wider internal tube design, whereas the cotton ball design could use 1.25 inch diameter pvc pipe and endcaps. Each design tested one of each of the following three baits: 25% sugar water, 25% sugar water + 5% hydrolyzed casein, and 25% sugar water + 5% sodium caseinate (see section 3 below for discussion of the use of casein additives to the baits).

I found that the cotton ball stations worked considerably better than the weedblock stations for the following reasons. 1) The vertical design of the weedblock stations necessitates some sort of additional mounting device, such as a wooden stake, in areas where stations cannot be mounted directly to trees or shrubs. This increases cost and makes checking/servicing the stations more difficult and time consuming. 2) Despite being held vertically, the weedblock leaked over time, probably more so as temperatures were warmer. The bait therefore leaked into the outer pvc tubes, creating puddles that drowned some ants, and leaked through the holes in the outer pvc endcaps to drip on the ground. This also led to greater exposure to the bait while handling the stations. 3) The vertical design caused the hydrolyzed casein precipitate to settle on the weedblock membrane, and this appeared to hinder bait uptake by ants after several days. 4) The weedblock design may require double the number of centrifuge caps, since one cap needs to have a hole drilled in it for access, and a second cap is highly convenient because it allows stations to be filled and processed in the lab and transported to the field without spilling. This would likely require the purchase of double the number of needed centrifuge tubes. 6) The weedblock design requires 1.5 inch pvc pipe and endcaps instead of 1.25 inch pvc, which increases the cost considerably.

I therefore chose the cotton ball internal delivery design for the present experiment. To minimize leakage/spillage inside the bait stations, a large cotton ball is important; most cotton balls available at local pharmacies and grocery stores are too small. I used large size Kendall Curity Prepping Balls (available on the internet), which tended to work well, although sometimes two of these balls were needed to properly retain the liquid bait. In addition, the bait stations need be placed at an incline of probably at least 20 degrees, which is not difficult in the uneven terrain at HALE, but may be more difficult in situations with predominantly flat ground. I used a 10 inch length of 1.25 inch diameter pvc pipe, combined with two 1.25 inch diameter smooth endcaps, for the external housing of the bait stations (Fig. 1). This size permits the insertion of two 50 ml plastic centrifuge tubes (Fisher or Corning brands) in each station if necessary, although some type of internal spacer would be required between the two centrifuge tubes to allow ant access to both. As in the bait station design test, four 5/64 inch holes were drilled in each endcap for ant access, and the exterior of the endcaps were roughened slightly with sandpaper to improve the climbing surface. Larger ant species, such as *Anoplolepis gracilipes*, might require slightly larger access holes in the endcaps. The cost of each station was approximately $3.10 using one internal tube, and $3.46 using two internal tubes. While fairly expensive, this design compares favorably with the Km AntPro bait station (approximately $12.50 each, although the Km AntPro station is more economical on a per volume of bait basis), and could potentially be made somewhat cheaper through bulk purchases with direct pvc suppliers.

Figure 1. Bait station design chosen for the experimental plots. The panel on the left shows placement of a bait station in the field, while the panel on the right shows a close-up view of one endcap (removed) with the four entrance holes, as well as an internal bait tube containing bait stoppered with a cotton ball.

To produce a rough estimate of the rate of evaporation with the chosen bait station design, I prepared two tubes each of 30 to 40 ml of 100% Gourmet Liquid Ant Bait and 95% Gourmet + 5% hydrolyzed casein. These four bait tubes were weighed and then placed in the field, within pvc stations using the cotton ball design, just outside the lower ant population at HALE, and were therefore not subject to ant foraging. (No other organisms were found inside the bait stations during this test.) I collected one tube of each formulation after one week; the 100% Gourmet tube gained 0.32 g, while the 95% Gourmet + 5% hydrolyzed casein tube lost 1.37 g. I collected the remaining two tubes after three weeks in the field; the 100% Gourmet tube lost 2.80 g, while the 95% Gourmet + 5% hydrolyzed casein tube lost 2.45 g. This is equivalent to 0.93 g per week for 100% Gourmet Ant Bait, and 0.82 g per week for the 95% Gourmet +5% hydrolyzed casein formulation. The bait stations therefore appear to lose approximately 1 g of water per week to evaporation. This loss equals about 6% of total bait weight after 3 weeks and about 8% of total bait weight after 4 weeks (assuming that each bait tube contains roughly 50 g of bait), which should have an insignificant effect on the concentration of the active ingredient.

3. BAIT FORMULATION AND ATTRACTIVENESS

A. Protein additives

Informal bait preference tests conducted in late February of 2007 with different dilutions of Gourmet Liquid Ant Bait as well as a 25% sucrose solution indicated that Argentine ant interest in sugar-based foods was very low at this point in time. This was consistent with a previous year-long bait preference test conducted at HALE, in which attractiveness of 25% sugar water was low in winter and increased markedly in May (Krushelnycky and Reimer 1998a). I therefore began experimenting with protein additives, and found that soy protein mixed in with a sucrose solution greatly increased the attractiveness of the liquid bait. This was followed by investigations using the mammalian milk protein casein, which can be obtained in purified form in large quantities, and which has been found to be attractive to other ants (e.g. *Solenopsis invicta*, Howard and Tschinkel 1981).

Casein can be prepared in a variety of formulations. I conducted a series of tests with the following casein products, obtained from American Casein Company, mixed into both 25% sugar water and Gourmet Liquid Ant Bait at concentrations ranging from 2 to 5% (by weight): hydrolyzed casein, sodium caseinate, partly hydrolyzed sodium caseinate, micellar casein, and instantized micellar casein. These casein products exhibited different solubilities in sugar water and in Gourmet Liquid Ant Bait, and different levels of attractiveness to ants when offered in choice preference tests in the field. Hydrolyzed casein had the best overall performance. It was consistently the most attractive casein product when added to both sugar water and Gourmet, and was the easiest to mix with the liquid baits. The largest drawback to hydrolyzed casein was the fact that much of the added powder precipitated out of solution after several hours. Sodium caseinate was the only casein product that was fully soluble in sugar water, however it was very difficult to produce a solution with a casein concentration higher than about 2 or 3% (by weight). Moreover, it appeared to be practically insoluble in Gourmet, possibly due to some chemical reaction with the borate toxicant or preservative, and it was consistently less attractive as an additive than was hydrolyzed casein. Partly hydrolyzed sodium caseinate had similar solubility problems in Gourmet. The two micellar casein products were also less attractive than hydrolyzed casein, but like the latter came out of solution after some period of time. For a protein additive, I used hydrolyzed casein exclusively in all subsequent tests and field trials because of its superior performance, and all further mention of casein in this report, if not specified, refers to the hydrolyzed casein formulation.

Finally, I conducted a preference test with casein to determine whether higher concentrations of casein additive are more attractive than lower concentrations. In late April, I set out five replicate arrays of bait stations in the lower Argentine ant population, each separated by at least 20 m and each containing three different bait formulations: 25% sugar water, 25% sugar water + 2% hydrolyzed casein, and 25% sugar water + 5% hydrolyzed casein. All bait tubes were weighed before the preference test and then again after four days in the field. A one-way ANOVA followed by a Tukey HSD test found that the loss in weight of the 5% casein baits was significantly greater than that of the 2% casein baits ($t = 3.44$, $p = 0.0125$) and the plain sugar water baits ($t = 4.06$, $p = 0.0042$) (Fig. 2). Despite the fact that most of the casein powder appeared to precipitate out of solution at both concentrations, the results of this test clearly indicated that sugar water formulated with 5% casein was more attractive than sugar water formulated with 2% casein or with no casein. I did not consider higher concentrations of casein because of the extra cost and because the 5% casein formulation already increased relative attractiveness so dramatically. I decided to use 5% casein (hydrolyzed form) in all subsequent formulations with Gourmet Liquid Ant Bait.

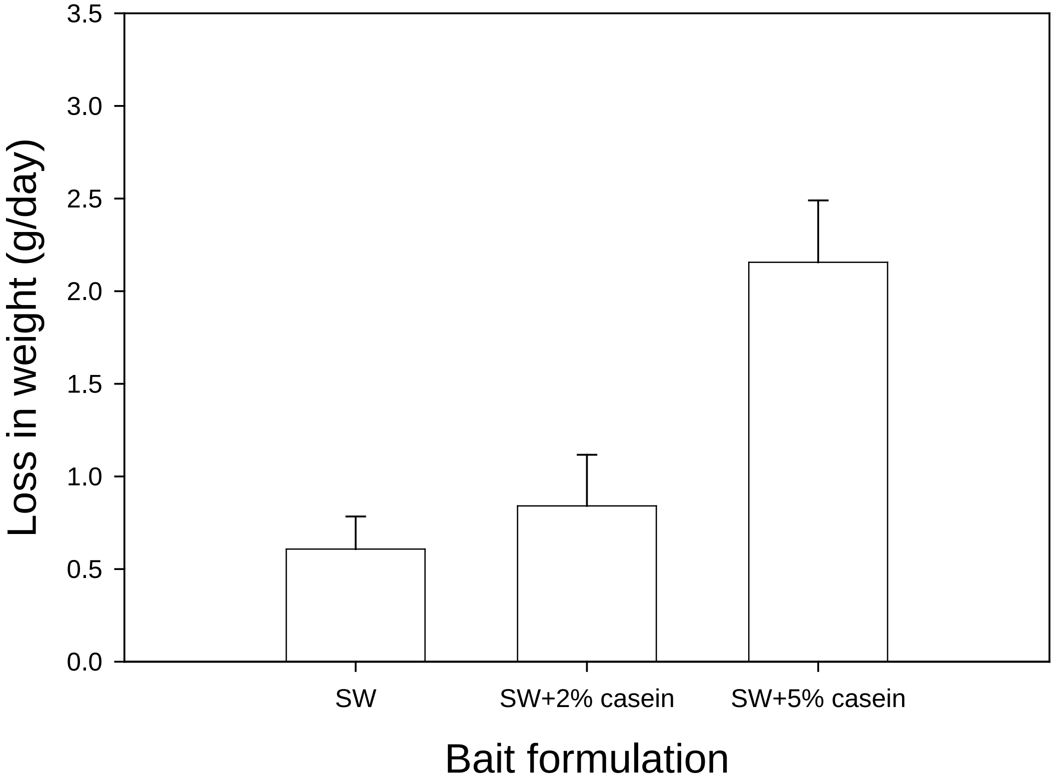

Figure 2. Mean loss in weight per day, over the course of four days, for three bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. SW is 25% sugar water, while formulations with casein used hydrolyzed casein (percent composition is by weight).

B. Gourmet Liquid Ant Bait attractiveness, part I

In April 2007, I found that a formulation of full strength Gourmet Liquid Ant Bait, with casein additive, was still relatively unattractive to Argentine ants at HALE (in comparison to sugar water and casein formulations). The owner and manufacturer of Gourmet (Innovative Pest Control Products) suggested that a dilution of the bait may be more attractive to the ants due to lower viscosity. A series of informal bait preference tests conducted in the field at HALE supported the idea that diluted Gourmet is more attractive, but the optimal dilution strength and diluting liquid (water versus sugar water) still needed to be determined. Similar to the bait preference test described above, I set out four replicate arrays of bait stations in the lower Argentine ant population on 5/1/07, each separated by at least 20 m and each containing four different bait formulations: 75% Gourmet + 5% hydrolyzed casein, diluted with water; 75% Gourmet + 5% hydrolyzed casein, diluted with 25% sugar water; 50% Gourmet + 5% hydrolyzed casein, diluted with water; and 50% Gourmet + 5% hydrolyzed casein, diluted with 25% sugar water. All bait tubes were weighed before the preference test and then again after four days in the field. A one-way ANOVA followed by a Tukey HSD test found that there was no significant difference in weight loss between any of these formulations (all comparisons, $p \geq 0.76$). However, 75% Gourmet + 5% hydrolyzed casein, diluted with 25% sugar water, had the highest mean weight loss (Fig. 3). This fact, along with the higher borate concentration relative to the 50% Gourmet formulations, led me to chose this formulation for the experimental ant control plots, at least initially (see below).

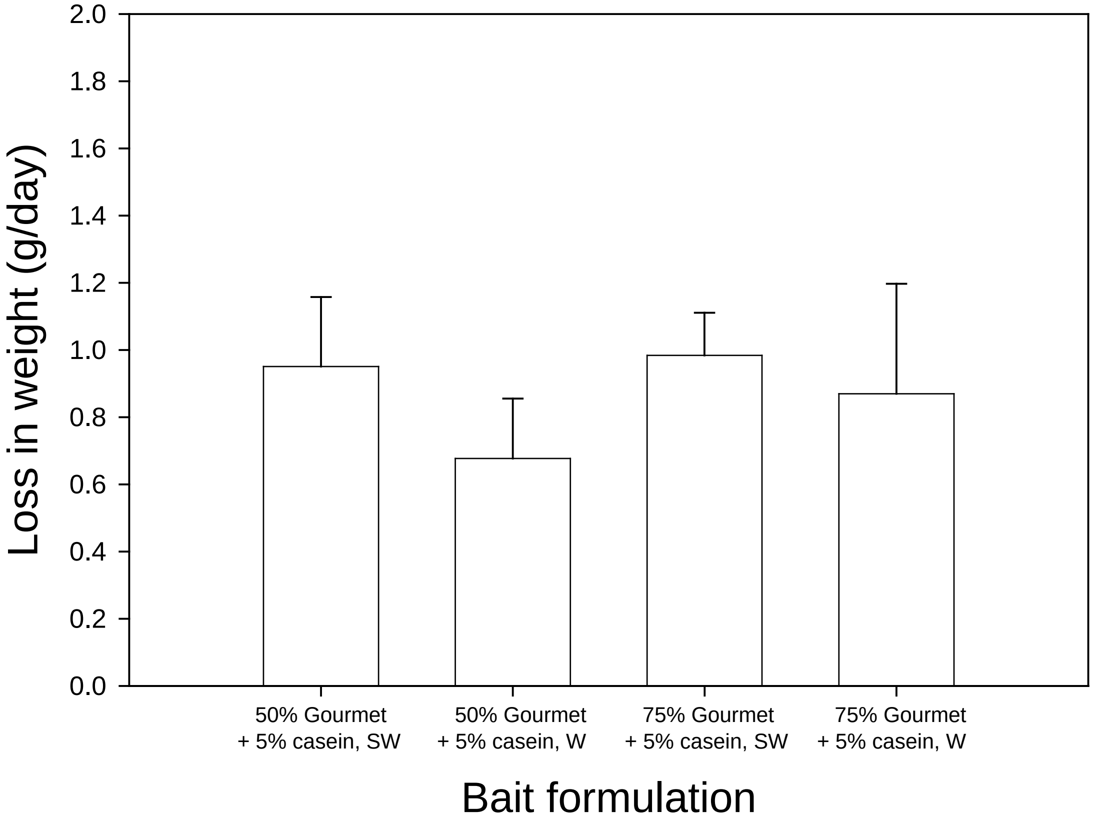

Figure 3. Mean loss in weight per day, over the course of four days, for four Gourmet Liquid Ant Bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. SW indicates formulations were diluted with 25% sugar water, while W indicates formulations were diluted with water.

Importantly, this bait preference test suggested that attractiveness of these Gourmet formulations was now similar to those of baits using 25% sugar water as the base, because total weight loss for each replicate group of stations was similar to that of the sugar water bait preference tests. The use of casein, combined with dilution, appeared to be critical to increasing attractiveness of the Gourmet bait. However, protein additives have the disadvantage of increasing rates of bait spoilage. I gauged the importance of this factor by observing molding in four tubes of Gourmet bait, two containing 5% casein and two with no casein, placed in the field (in an area with no ants) within bait stations for three weeks. After three weeks, the two baits lacking casein additives showed no signs of molding or bait spoilage. The two baits containing 5% casein also had no visible mold. The liquid in these tubes did become somewhat discolored, appearing more yellow than when initially placed in the field, however they did not have a noticeable odor that might indicate spoiling. In previous tests, it was observed that ants often continued to forage heavily on sugar water and casein baits even after substantial amounts of mold began to grow on the cotton plug. Based on these results, it appeared that Gourmet formulated with casein would probably last in the field for at least three to four weeks.

C. Gourmet Liquid Ant Bait attractiveness, part II

Despite the promising results of the final bait preference tests reported in section B. above, attractiveness of the initial Gourmet formulation chosen for the experimental ant control plots (75% Gourmet + 5% casein, diluted with sugar water) was low when used in the plots (see section 4.B below). I therefore investigated additional bait modifications that might increase Gourmet attractiveness. I suspected that the preservative used in Gourmet Liquid Ant Bait might be decreasing attractiveness, and the owner of Innovative Pest Control Products suggested that adjustment of the pH of the bait formulation, or dilution of the bait with fruit juice, might increase bait attractiveness at HALE.

Preservative

Gourmet Liquid Ant Bait uses 0.25% sodium benzoate (by weight) for a preservative. I tested the effect of sodium benzoate on bait attractiveness by comparing consumption of the following two baits: 25% sugar water + 5% hydrolyzed casein + 0.75% boric acid + 0.25% sodium benzoate, and 25% sugar water + 5% hydrolyzed casein + 0.75% boric acid. The baits were therefore identical except for the inclusion of sodium benzoate; boric acid was added to each bait to approximate the concentration of borates in a 75% Gourmet bait solution. I set out five replicate pairs of the two baits in bait stations in the lower Argentine ant population on 6/13/07, each separated by at least 20 m. The bait tubes were weighed before the preference test and then again after three days in the field. The bait without sodium benzoate was clearly more attractive (one-way ANOVA and Tukey HSD test, $t = 3.14$, $p = 0.02$) than the same bait with 0.25% sodium benzoate (Fig. 4). These results indicated that the preservative likely has some negative effect on the attractiveness of Gourmet. However, some type of preservative is likely to be necessary, especially when protein is added to the bait. Further dilutions of the bait, along with additions of fruit juice, could minimize the negative effect of the preservative.

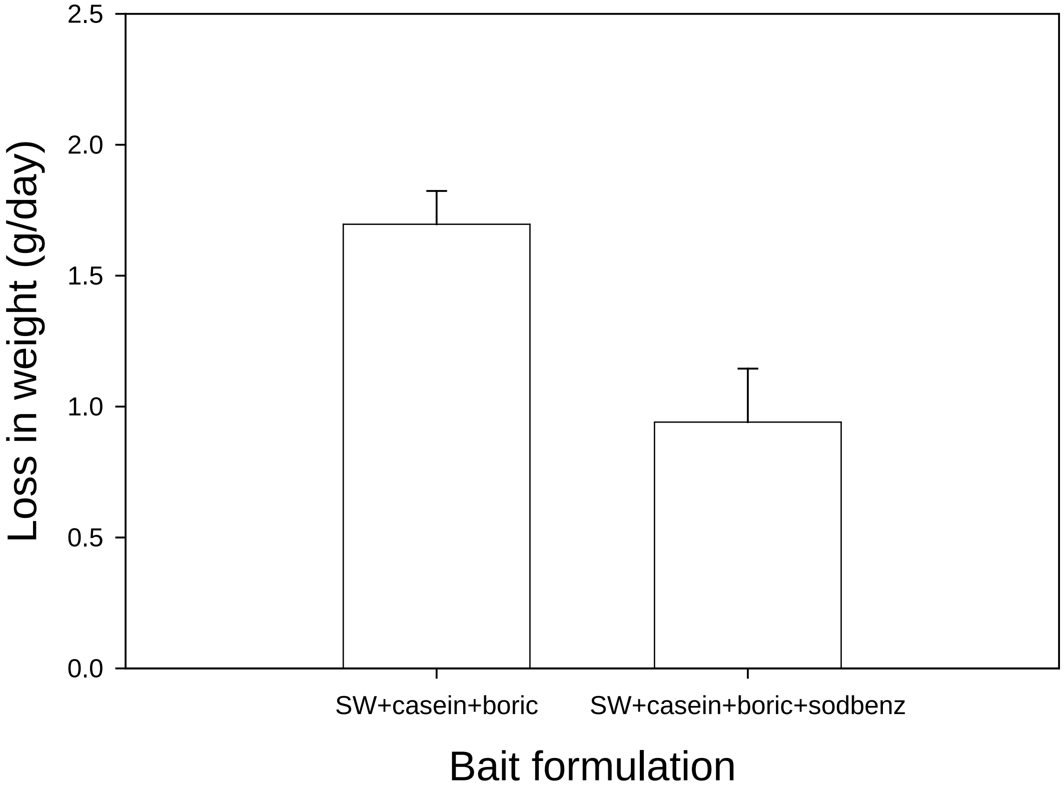

Figure 4. Mean loss in weight per day, over the course of three days, for two sugar water based bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. SW indicates 25% sugar water, casein indicates the addition of 5% hydrolyzed casein (by weight), boric indicates the addition of 0.75% boric acid (by weight), and sodbenz indicates the addition of 0.25% sodium benzoate (by weight).

The effect of pH

I next investigated the effect of pH on bait attractiveness. I measured the pH of a variety of sugar water, Gourmet and other formulations with a Hach sension3 pH meter (Table 1). These measurements yielded several interesting findings. First, the 100% Gourmet bait was more acidic than I anticipated, and even the diluted formulations (6-9) were fairly acidic. Also surprising was the high pH of the park's tap water (15), which was used for all dilutions and sugar water solutions. Second, certain additives didn't always have consistent effects on pH. Sodium benzoate had a moderate effect on pH when added to 25% SW + 1.2% boric acid (formulation 19 versus 20), but didn't have an effect when added to 25% SW + 0.75% boric acid + 5% casein (3 versus 4). In addition, casein lowered pH when added to 25% sugar water (1 versus 2), but raised pH when added to 75% Gourmet diluted with 25% sugar water (6 versus 13) or 50% Gourmet diluted with water (9 versus 14). Chemical reactions between borates, sodium benzoate and casein may be responsible for these inconsistencies. Alternatively, variation in the pH of the tap water used in the various formulations could have been responsible for some of the inconsistencies. For example, two identical 25% sugar water solutions mixed at different points in time (1 and 16) differed substantially in pH.

Table 1. pH measurements of a variety of potential liquid bait formulations and standards.

Formulation	pH
1. 25% sugar water (SW)	7.78
2. 25% SW + 5% hydrolyzed casein (casein)	6.82
3. 25% SW + 5% casein + 0.75% boric acid + 0.25% sodium benzoate	6.51
4. 25% SW + 5% casein + 0.75% boric acid	6.50
5. 100% Gourmet	4.59
6. 75% Gourmet, diluted with 25% SW	4.83
7. 75% Gourmet, diluted with water	4.87
8. 50% Gourmet, diluted with 25% SW	5.17
9. 50% Gourmet, diluted with water	5.24
10. 75% Gourmet, diluted with orange juice	4.68
11. 50% Gourmet, diluted with orange juice	4.67
12. 100% orange juice	3.90
13. 75% Gourmet + 5% casein, diluted with 25% SW	5.20
14. 50% Gourmet + 5% casein, diluted with water	5.67
15. tap water	10.24
16. 25% sugar water (batch #2)	8.87
17. 25% SW + 0.5% boric acid	5.50
18. 25% SW + 0.75% boric acid	5.30
19. 25% SW + 1.2% boric acid	5.05
20. 25% SW + 1.2% boric acid + 0.25% sodium benzoate	5.57
21. distilled water from maintenance water quality lab	8.75

Because acidity of the Gourmet baits may have decreased their attractiveness, I measured the ability of a basic food-grade additive, sodium bicarbonate (baking soda), to raise the pH of a Gourmet bait formulation (Table 2). Based on these measurements, I chose three formulations using sodium bicarbonate for a bait preference test in the field: 50% Gourmet + 5% casein, diluted with water (approx. pH = 5.65); 50% Gourmet + 5% casein + 0.25% sodium bicarbonate, diluted with water (approx. pH = 6.10); and 50% Gourmet + 5% casein + 1.0% sodium bacarbonate, diluted with water (approx. pH = 6.85). I set out five replicate groups of the three baits in bait stations in the lower Argentine ant population on 6/22/07, each separated by at least 20 m. The bait tubes were weighed before the preference test and then again after four days in the field. Although there appeared to be slightly higher bait loss with the 1% sodium bicarbonate formulation as compared to the formulations with 0.25% sodium bicarbonate or no sodium bicarbonate (Fig. 5), these differences were not statistically significant (one-way ANOVA and Tukey HDS test, p = 0.56 and p = 0.51, respectively). Moreover, the addition of sodium bicarbonate appeared to lower the surface tension of the bait formulations, causing more bait to seep through the cottom ball in the bait tubes. This often lead to high numbers of drowned ants and/or bait spillage inside the bait stations. Subsequent tests with higher concentrations of sodium bicarbonate resulted in more bait spillage, and this negative effect on overall bait performance was judged to outweigh any potential benefits to bait attractiveness gained through raising bait pH (which appeared to be slight, at best). I did not investigate other additives that could raise the pH of the bait.

Table 2. The effect of sodium bicarbonate on the pH of a potential Gourmet bait formulation.

Formulation	pH
1. tap water	10.22
2. 50% Gourmet + 5% casein, diluted with water	5.65
3. 50% Gourmet + 5% casein, diluted w/ water, + 0.25% sodium bicarbonate	6.10
4. 50% Gourmet + 5% casein, diluted w/ water, + 0.5% sodium bicarbonate	6.44
5. 50% Gourmet + 5% casein, diluted w/ water, + 0.75% sodium bicarbonate	6.70
6. 50% Gourmet + 5% casein, diluted w/ water, + 1.0% sodium bicarbonate	6.85
7. 50% Gourmet + 5% casein, diluted w/ water, + 1.5% sodium bicarbonate	7.02
8. 50% Gourmet + 5% casein, diluted w/ water, + 2.0% sodium bicarbonate	7.10
9. 50% Gourmet + 5% casein, diluted w/ water, + 4.0% sodium bicarbonate	7.43

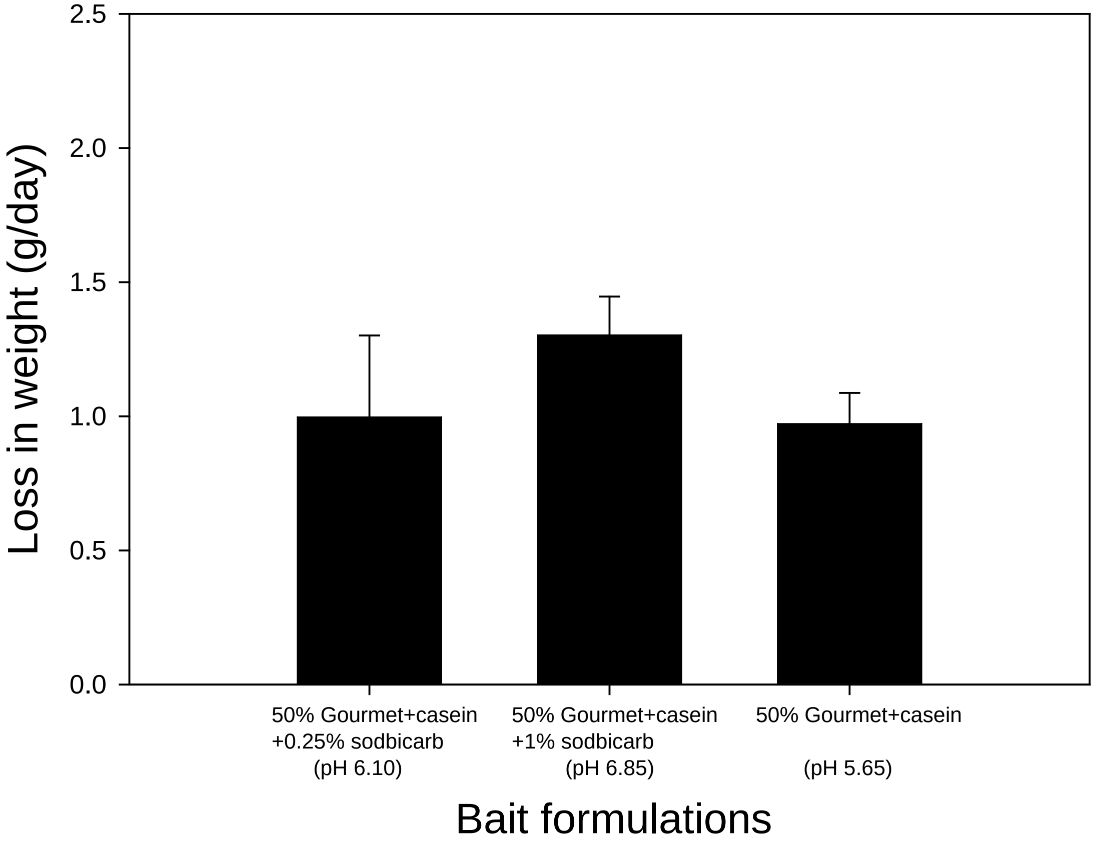

Figure 5. Mean loss in weight per day, over the course of four days, for three Gourmet bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. Casein indicates the addition of 5% hydrolyzed casein (by weight), and sodbicarb indicates the addition of sodium bicarbonate (percent by weight).

Dilution with fruit juice

In a final attempt to improve Gourmet bait attractiveness, on the advice of Innovative Pest Control Products I tested the effect of mixing Gourmet with different fruit juices. I diluted the Gourmet bait roughly 1:1 with four commonly available fruit juices (orange juice, grape juice (dark grapes), cranberry juice and apple juice) because the 25% dilution originally used in the field experiment proved to be relatively unattractive. I continued to add casein because it clearly increased bait attractiveness. The formulations tested, therefore, were 50% Gourmet + 5% casein + 45% fruit juice (all percentages by weight). I set out four replicate groups of the four formulations in bait stations in the lower Argentine ant population on 7/2/07, each group separated by at least 20 m. The bait tubes were weighed before the preference test and then again after three days in the field. The bait with grape juice had the highest mean weight loss (Fig. 6), however there were no significant differences between any of the juice formulations (one-way

ANOVA and Tukey HSD test, all comparisons $p \geq 0.94$). Also, each of the juice formulations except orange juice was the favorite in at least one replicate group, indicating that there was no strong preference for one particular juice, and that they were all relatively attractive.

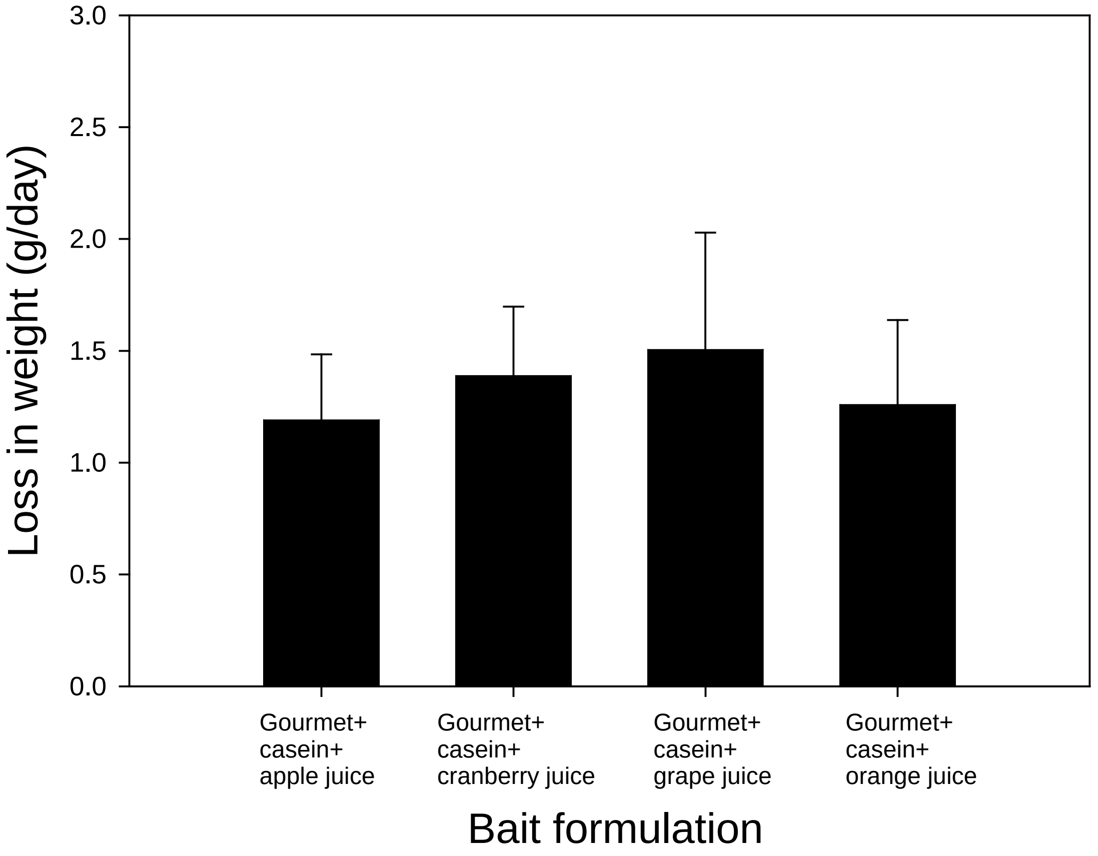

Figure 6. Mean loss in weight per day, over the course of four days, for four Gourmet and fruit juice bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. All formulations were 50% Gourmet + 5% casein + 45% fruit juice (all percentages by weight).

The mean total weight loss of bait in each replicate group was higher in this last bait preference test (5.34 g/day) than in any previous bait preference test. This may have been due, at least in part, to increasing abundances of ants as the summer progressed, but it also may have indicated greater attractiveness of the fruit juice bait formulations. Moreover, the addition of fruit juice did not detract from bait performance, in contrast to either foregoing the preservative (which would decrease the life of the bait in the field) or neutralizing the bait’s acidity with sodium bicarbonate (which causes greater bait spillage and ant drowning). Because of the low attractiveness of the initial bait formulation (75% Gourmet + 5% hydrolyzed casein, diluted with 25% sugar water; see section 4.B below) when deployed in the experimental plots, I chose to replace it on 7/10/07 with the fruit juice formulation that appeared to be the most attractive (if only slightly) of the four tested: 50% Gourmet + 5% hydrolyzed casein + 45% grape juice.

4. EXPERIMENTAL PLOTS

Because the two Argentine ant populations at HALE are so large, any eradication strategy that uses numerous bait stations would likely have to work incrementally from the periphery of the populations to their centers. This periphery-to-center approach would focus resources on smaller border areas at any one time and should therefore increase the chances of success. Further outward spread of the ant populations would concurrently be prevented. I therefore chose to position the experimental eradication plots testing the effectiveness of Gourmet Liquid Ant Bait along an ant population border. The plots would thus simultaneously evaluate the effectiveness of this bait for Argentine ant population containment and eventual eradication. I also chose to test two different bait station spacing intervals, and hence application rates, since effort and cost greatly decrease as bait station interval increases.

A. Methods

Plot layout

I established three experimental plots along the boundary of the lower Argentine ant population in HALE to test the effectiveness of Gourmet Liquid Ant Bait for eradicating ants from the park. Two of the plots were randomly designated treatment plots, while the third plot served as a non-treated control. The two treatment plots were 140 m wide by 120 m deep, and were situated such that 100 m of the depth of each plot extended behind the ant population boundary and 20 m extended ahead of the boundary as a buffer (Fig. 7). Each treatment plot contained a 60 m by 60 m central monitoring core; this monitoring core was therefore surrounded by a 40 m buffer provisioned with pesticidal bait stations designed to prevent the spread of untreated nests from outside the plots into the monitoring area. The large size of the buffer zones (and therefore overall treatment plots) was deemed especially important in this experiment because borates act more slowly than most insect toxicants, and hence recolonizing nests would in theory be suppressed relatively slowly within the buffer zones. The control plot replicated the design of the monitoring cores of the treatment plots and was therefore only 60 m by 60 m in size (Fig. 7).

Bait application

Bait stations were deployed in one of the treatment plots in a 10 m grid pattern (195 bait stations), and were deployed in the second treatment plot in a 20 m grid pattern (56 bait stations). The control plot contained no bait stations. I used the bait station design described in section 2 above (see also Fig. 1). Baits were first deployed in the stations in the two treated plots on 5/7/08-5/11/07. The original bait formulation for this application was 75% Gourmet + 5% hydrolyzed casein (by weight), diluted with 25% sugar water (see section 3.B above). Each station received 45 ml of this formulation in a single centrifuge tube stopped with a cotton ball.

Because the original bait formulation attracted relatively few ants to the stations (see section 4.B below), I replaced the bait in all stations in both treatment plots on 7/10/07-7/11/07 with a new formulation: 50% Gourmet + 5% hydrolyzed casein + 45% Welch's dark grape juice (by weight) (see section 3.C above). Each station again received 45 ml of bait in a single centrifuge tube stopped with a cotton ball. Although few stations were ever emptied of bait due to consumption by ants, I replaced the bait in all stations in both plots with fresh bait (using the second formulation) on 9/9/07-9/10/07, and again on 10/23/07-10/25/07. For the final refill in October 2007, each bait station received 40 ml of bait instead of 45 ml.

Monitoring

I conducted four types of monitoring: bait station monitoring to assess the attractiveness and/or rate of consumption of the Gourmet bait, bait card monitoring (using non-toxic attractants) to assess relative ant abundance levels in the plots, nest surveys to assess survival of queens and immature stages in the plots, and spread monitoring along transects to measure the rate at which the ant population boundary spread outward at each plot.

At periodic intervals after initial bait placement, I visited a subset of the bait stations in each treated plot (between 20 and 60 stations on each occasion) and recorded the approximate number of ants in each station (according to abundance class: 0, 1-20, 21-50, >50), the approximate volume of bait remaining in the station, and the degree of mold on the cotton balls in each station (0 = none; 1 = small amount; 2 = medium amount; 3 = large amount). For the last three bait refills, I also weighed the bait tubes when they were removed from the plots to estimate the amount lost to consumption and/or evaporation. This was done by comparing weights of all baits removed from the plots with the average weight of fresh tubes of bait prior to placement in the plots (average calculated from 25 tubes containing 45 ml of fresh bait, and 25 tubes containing 40 ml fresh bait in the case of the last bait refill event).

The bait card monitoring and the nest surveys were conducted in the central 60 m by 60 m monitoring core of each plot. Each monitoring core (and the entire control plot) was divided into 36 10 m by 10 m quadrats (Fig. 7). A single bait card was placed in the center of the 16 rear-central quadrats for the purposes of the bait card monitoring (Fig. 7). During each monitoring event, I provisioned each bait card with about 1.5 g of a blend of 40% tuna (in water) and 60% light corn syrup, by weight, and placed the bait card on the ground and in the shade for a period of 60 minutes. At the end of 60 minutes, I counted the number of ants on each card. Bait card monitoring was conducted on 5/1-5/3/07 (pre-treatment), and approximately every month after the initial bait placement until the termination of the experiment on 12/10/07-12/11/07, 32 weeks after initial bait placement.

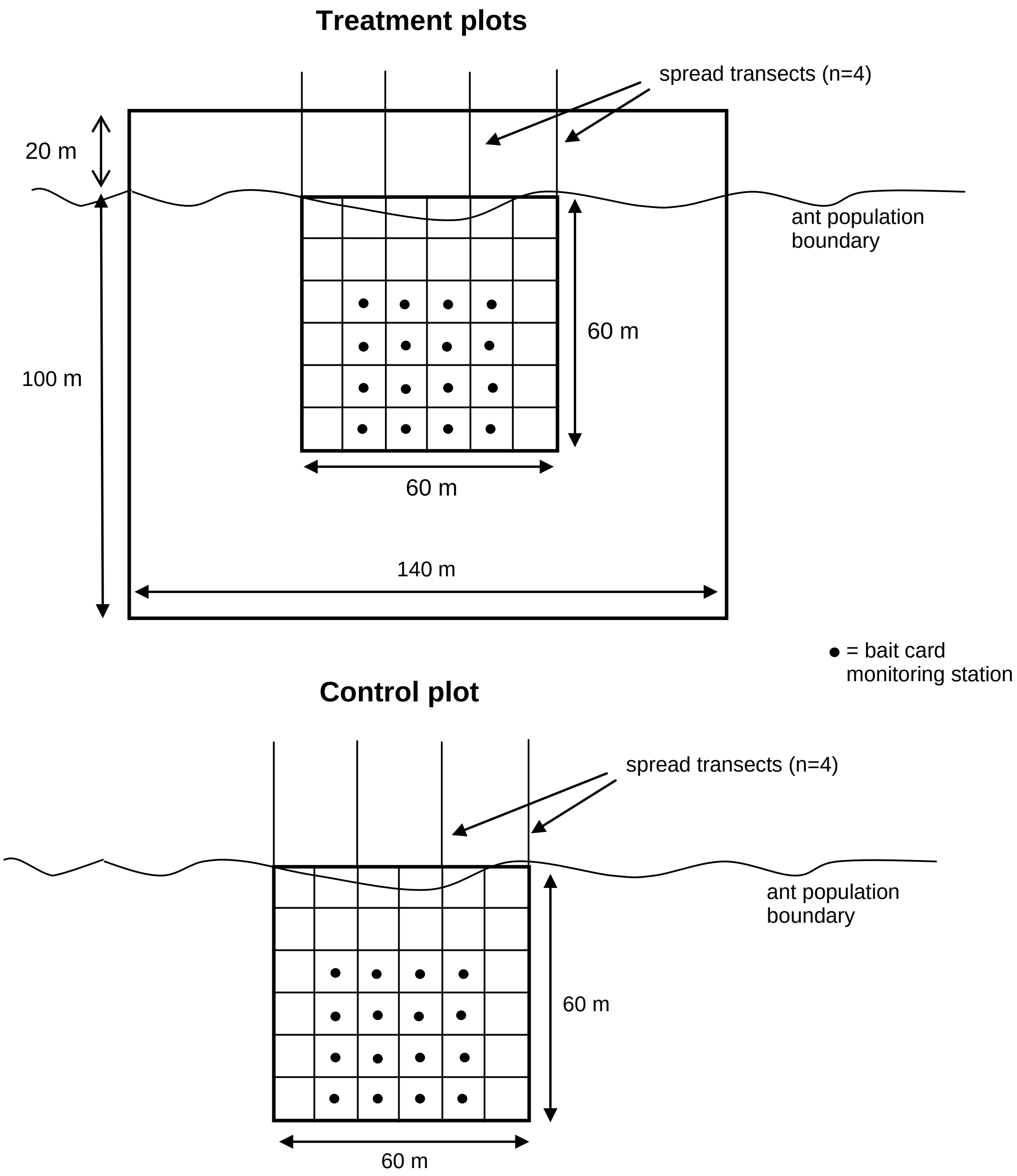

Figure 7. Plot layouts for the two treatment and one control plots. The central monitoring cores in each plot were divided into 36 10 m by 10 m quadrats for nest monitoring. Sixteen bait card monitoring stations in each plot are indicated with dots. Four spread monitoring transects emanated from each plot. Gourmet bait station locations are not indicated; stations were spaced in a 10 m grid pattern in one treatment plot (n=195 stations) and in a 20 m grid pattern in the second treatment plot (n=56 stations). The control plot received no Gourmet bait stations.

At the same monthly intervals, I randomly selected one of the 10 m by 10 m quadrats from the rear two rows in the central monitoring core of each plot (i.e. furthest from the ant population boundary, where ant densities are higher) for nest surveying. During each monitoring event, every rock in the selected quadrat was overturned in search of nests. All nests within the quadrat were recorded as reproductive (presence of queens, eggs, larvae or pupae) or nonreproductive (presence only of workers or males; or evidence of prior use as a nest site, such as presence of nest galleries). Numbers of individuals in each caste/stage were recorded, according to abundance categories: 0 = 0, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 101-500, 5 = >500. Nest surveys were conducted between 9:00 am and 12:00 pm on warm sunny days, when ants bring brood up to the soil surface (underneath the cover rock) where temperatures are warmer. Surveys were occasionally conducted slightly later on cooler days.

Finally, I monitored the rate of outward spread of ants along four parallel transects extending beyond each plot boundary. The transects were located 20 m apart in the central portion of each plot, starting at the ant population boundary, and oriented roughly perpendicular to the population boundary (Fig. 7). The exact position of the ant population boundary on each transect was marked prior to the initial bait placement, from 5/2/07-5/4/07. The furthest extent of ants along each transect was then mapped again at roughly one month intervals, during the same monitoring events that included bait card monitoring and nest surveys. These positions were mapped by searching the ground for foraging ants along each transect (and up to 5 m on either side of the transect) for 20 minutes.

B. Bait attractiveness in the plots

On all of the occasions that bait stations were monitored in the plots, there was no evidence of a high level of bait attractiveness. In separate bait preference tests conducted in the field (see section 3 above), highly attractive baits typically had a constant stream of ants entering and exiting the bait stations as well as at least 100 ants (and often many more) within the stations at any one time. While the experimental plots sometimes had a relatively high incidence of at least some ant presence in the bait stations (Table 3), this was usually represented by one to several individual ants. There was a much lower incidence of bait stations containing at least 20 ants in either plot, only exceeding 50% of stations during one monitoring event (on 9/21/07; Table 3). Only 11 bait stations were observed with over 50 ants inside them during the entire experiment, representing just 2.3% of all bait stations monitored. These results indicate that while ants successfully found most or all of the bait stations, the baits were not attractive enough to trigger the kind of mass recruitment seen with some of the baits in prior preference tests. This was true for the initial formulation chosen for the experimental plots (75% Gourmet + 5% hydrolyzed casein, diluted with 25% sugar water), as well as the second formulation that was adopted in an effort to improve field attractiveness (50% Gourmet + 5% hydrolyzed casein + 45% grape juice). Although ant presence within the bait stations appeared to increase somewhat in September and October (Table 3), this may have been due to an increasing abundance of ants in the environment during the fall months (Krushelnycky et al. 2004), rather than an increase in bait attractiveness.

Table 3. Attractiveness of Gourmet baits in the experimental plots, based on incidence of ants in the bait stations. All percentages greater than or equal to 50 are highlighted with bold typeface.

			20m grid plot		10m grid plot	
Date[1]	Form[2]	#days post placement[3]	% stns w/ ants[4]	% stns w/ >20 ants[5]	% stns w/ ants[4]	% stns w/ >20 ants[5]
5/17-5/18	1st	10	37.5	10	**63.3**	3.3
5/28-5/29	1st	18-21	42.5	2.5	36.7	2.5
6/18	1st	42	5	0	--	--
7/16	2nd	5-6	12.5	0	33.3	0
9/12-9/13	2nd	3	**50.0**	12.5	**66.7**	33.3
9/21	2nd	11-12	**70.8**	**62.5**	**65.4**	26.9
10/4	2nd	24-25	**75.0**	37.5	38.5	5.1

[1] Dates are expressed as month/day; all dates were in 2007

[2] Formulation in use at the time: 1st = 75% Gourmet + 5% hydrolyzed casein (by weight), diluted with 25% sugar water; 2nd = 50% Gourmet + 5% hydrolyzed casein + 45% Welch's dark grape juice (by weight)

[3] Number of days since the most recent refill of fresh bait

[4] Percent of stations monitored that had any ants inside them

[5] Percent of stations monitored that had at least 20 ants inside them

During these bait station monitoring events, I rarely observed mold growing on the cotton balls of the bait tubes, and there was no strong evidence that the baits used were substantially more attractive soon after fresh refills than several weeks after placement. In addition, bait stations were usually mostly full during monitoring events or at the time of bait replacement, indicating little bait consumption by ants. This judgement was confirmed during the second half of the experiment when bait tubes removed from the plots at the time of replacement were weighed to determine the amount of bait loss. Average weight loss of bait per day in the experimental plots was much lower than that which occurred in the preceding bait preference tests (compare Table 4 with Figs. 2-6). For example, in the final bait preference test examining attractiveness of Gourmet formulated with different fruit juices (section 3.C), ants consumed an average of over 1.5 g/day (Fig. 6) of the same formulation (50% Gourmet + 5% hydrolyzed casein + 45% grape juice) used in the plots and assessed in Table 4. Moreover, ants were presented with a side-by-side choice of 4 baits in each replicate location during the bait preference test, and therefore consumed an average of over 5.3 g of bait per day in each replicate location during the preference test. This compares with an average consumption (weight loss) of less than 0.5 g of bait per day in the experimental plots, regardless of whether bait stations were spaced at 10 m or 20 m intervals (Table 4). The degree of bait weight loss in the experimental plots decreased over time (Table 4), and during the last time period (10/23-12/13/07) was similar to that expected from evaporation alone (evaporative loss is approximately 0.12-0.20 g/day; see section 2).

Table 4. Mean weight loss of Gourmet bait per bait station per day during the latter half of the experiment.

Date[1]	Formulation[2]	20m grid plot mean weight loss (g/day)[3]	10m grid plot mean weight loss (g/day)[3]
7/10-9/10	2nd	0.41	0.43
9/9-10/25	2nd	0.32	0.23
10/23-12/13	2nd	0.13	0.10

[1] Time period for which weight loss was calculated. Dates are expressed as month/day; all dates were in 2007.
[2] Formulation in use at the time: 1st = 75% Gourmet + 5% hydrolyzed casein (by weight), diluted with 25% sugar water; 2nd = 50% Gourmet + 5% hydrolyzed casein + 45% Welch's dark grape juice (by weight).
[3] Mean weight loss of bait per bait station during each time period.

C. Effects on ant numbers and nest survival

The low attractiveness of the Gourmet bait formulations when used in the experimental plots translated into a low level of ant control. Densities of foraging ants in the two treatment plots, as inferred from bait card monitoring, appeared to be somewhat lower than in the control plot during the latter half of the experiment (Fig. 8), but for the most part population trends in the three plots were similar and mainly represented seasonal changes in density (e.g. see Krushelnycky et al. 2004). There was little evidence that ant densities were approaching zero in either treatment plot.

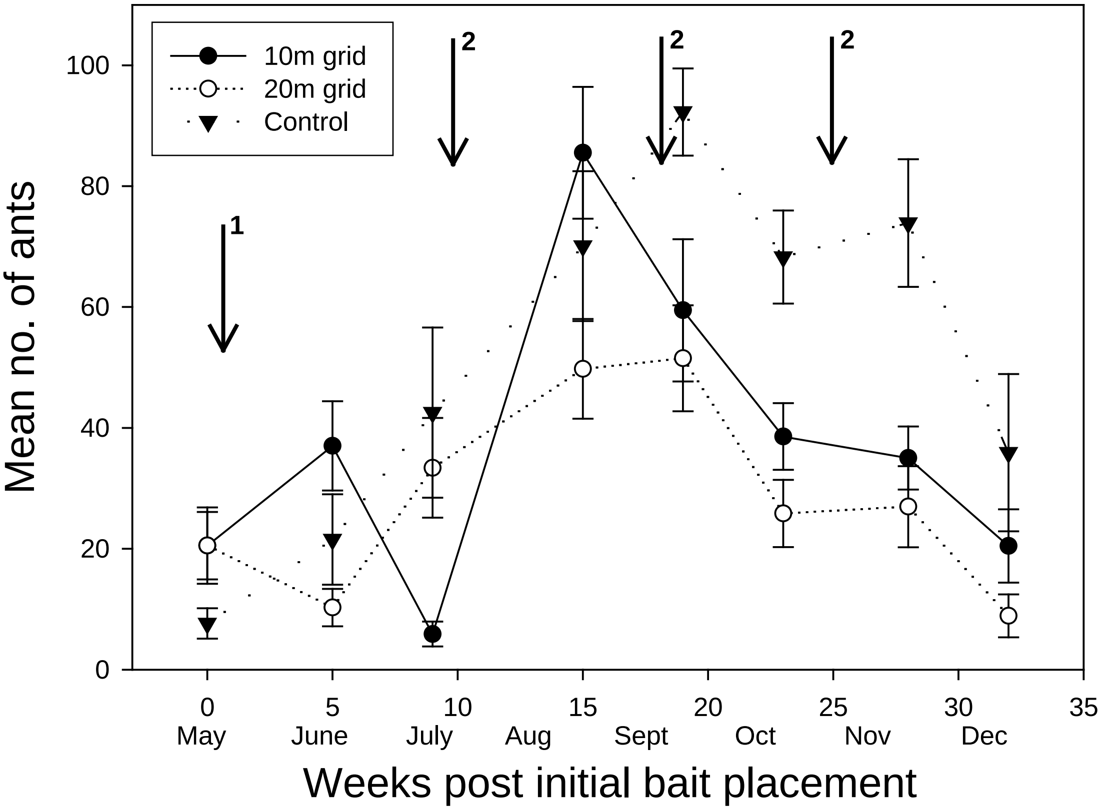

Figure 8. Mean number of ants at bait card monitoring stations in each plot. Bars indicate one standard error. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time (1 = 75% Gourmet + 5% hydrolyzed casein (by weight), diluted with 25% sugar water; 2 = 50% Gourmet + 5% hydrolyzed casein + 45% Welch's dark grape juice (by weight).

Nest survey data need to be interpreted with caution due to the high natural spatial variability in nest density. Because a different quadrat was surveyed in each plot during each monitoring event, differences between monitoring events in densities of nests or abundances of particular castes or life stages potentially represent natural spatial differences as much as or more than they represent temporal trends in these metrics. Elucidating subtle temporal and/or treatment-induced effects in the nest survey data is therefore difficult. However, nest surveys are highly effective for confirming dramatic effects (or a lack thereof), such as apparent eradication or high levels of control resulting from pesticide treatments. As can be seen in Figures 9-12, eradication was clearly not achieved in either treatment plot. Reproductive nests as well as eggs and worker larvae were present throughout the experiment, and their abundances in the treatment plots tended to be similar to or higher than those in the control plot. Queen presence was more variable in all plots (Fig. 10) due to the unpredictability of observing this caste. In general, the nest surveys corroborated the conclusion apparent from bait card monitoring of worker densities: the Gourmet formulations yielded little control in the two treatment plots.

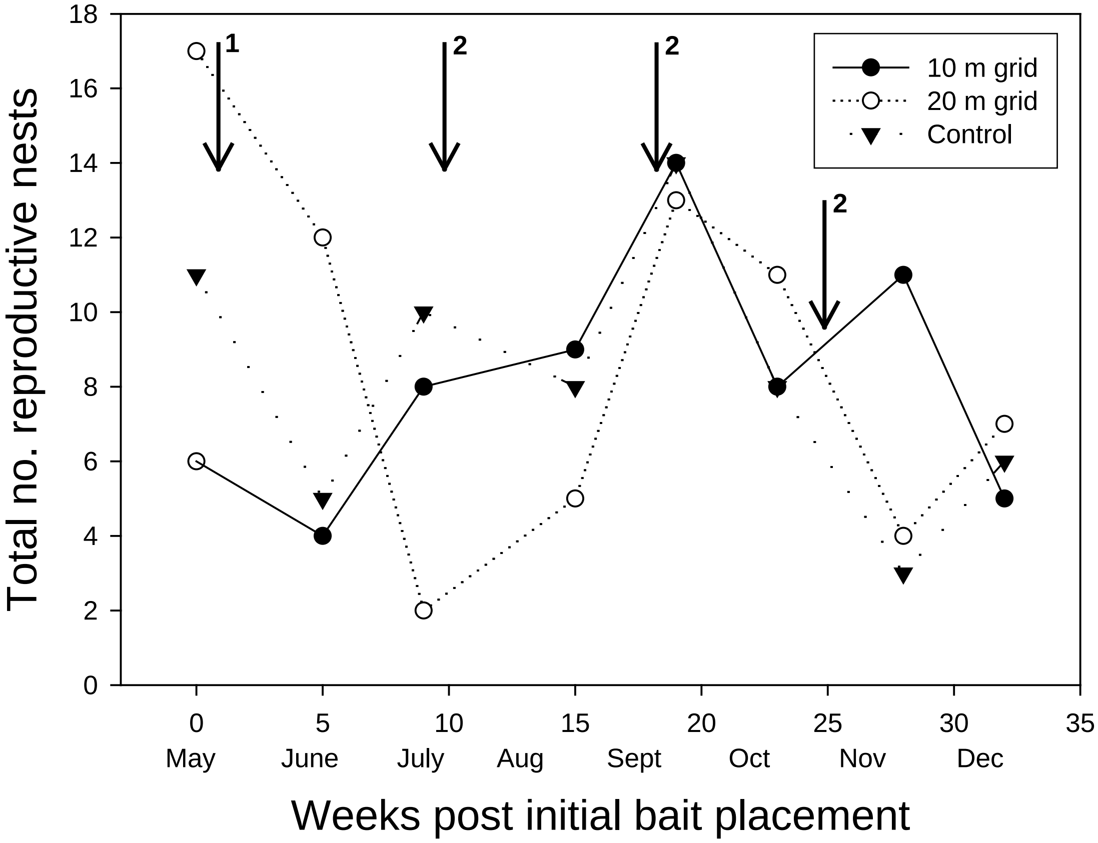

Figure 9. Total number of reproductive nests per monitoring quadrat in each plot. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time (same as in Figure 8).

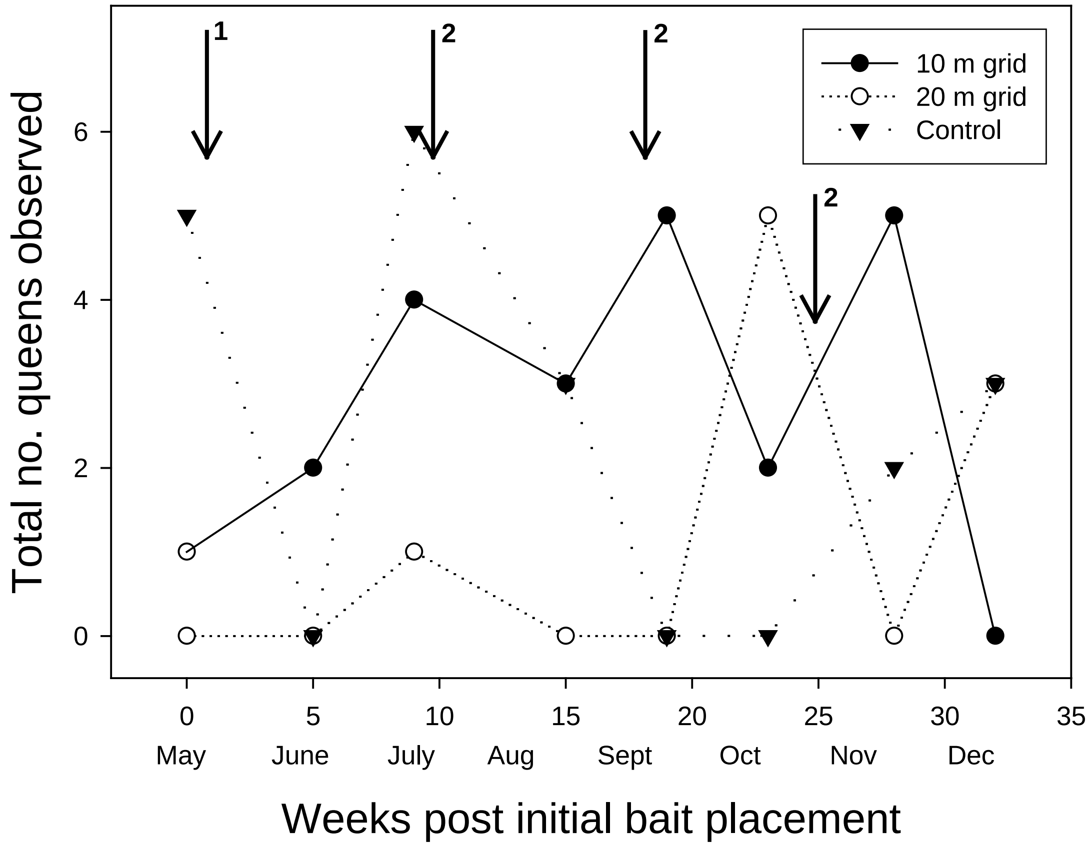

Figure 10. Total number of queens observed per monitoring quadrat during nest surveys in each plot. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time (same as in Figure 8).

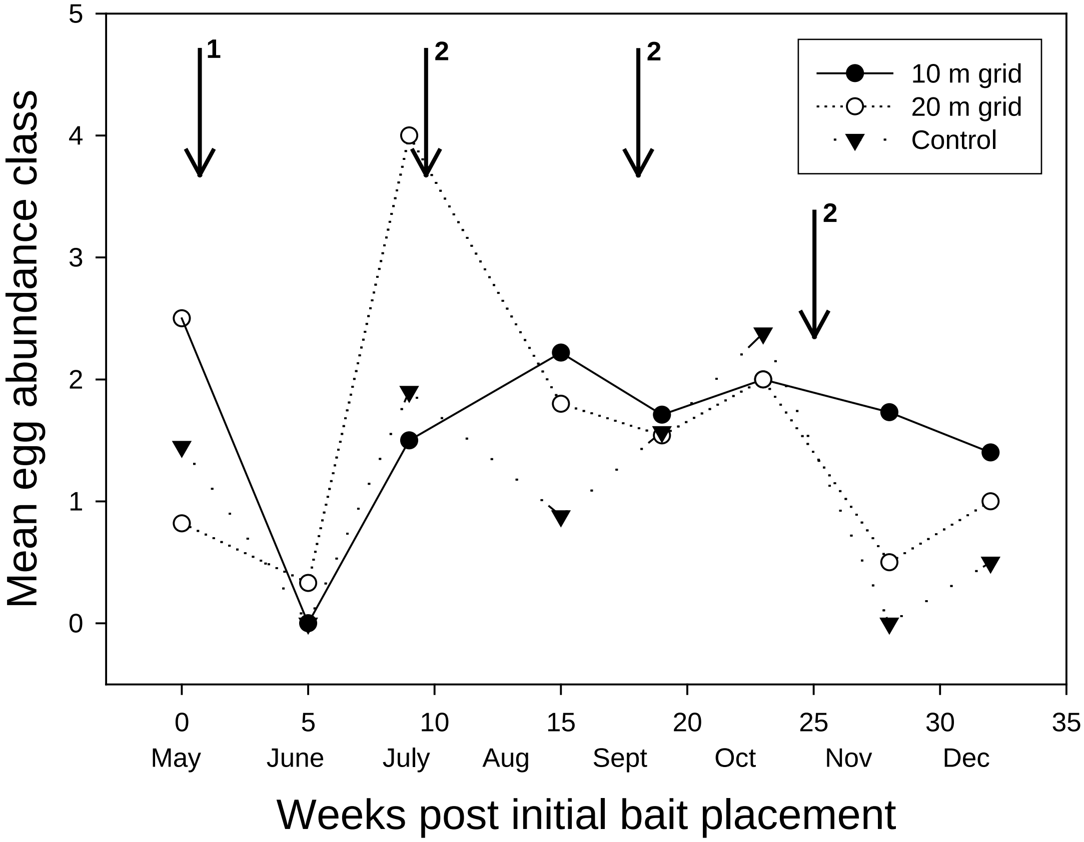

Figure 11. Mean egg abundance class of reproductive nests in each plot. Abundance classes are 0 = 0, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 101-500, 5 = >500. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time (same as in Figure 8).

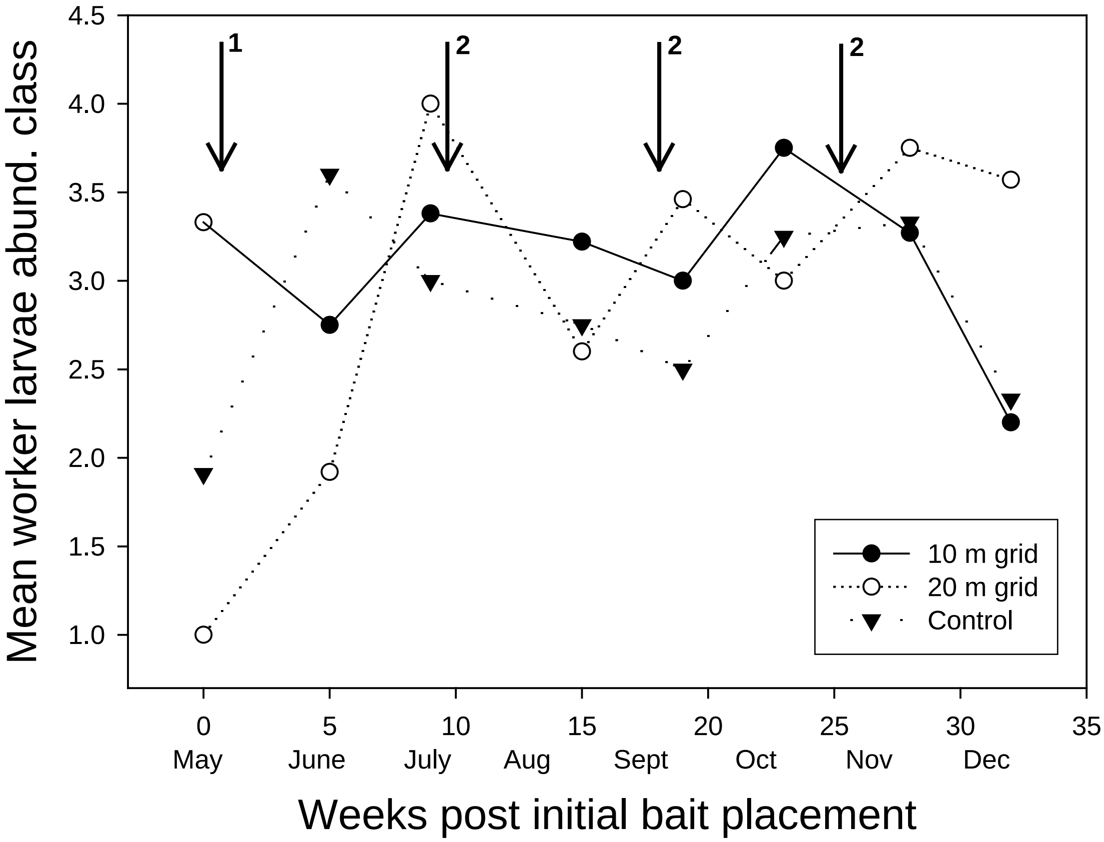

Figure 12. Mean worker larvae abundance class of reproductive nests in each plot. Abundance classes are 0 = 0, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 101-500, 5 = >500. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time (same as in Figure 8).

D. Effects on rate of ant spread

The lower ant population boundary spread outwards from the border of the control plot a total of 39.6 m (mean of four transects) during the course of the experiment, from early May to mid-December 2007. Most of this spread occurred from May through September (Fig. 13), and therefore approximated the seasonal pattern of spread measured in the same general area during 1996-97 (Krushelnycky et al. 2004). In 1996-97, however, the peak period of spread was slightly later, with most occurring from July through October, and total spread during the months of May through December was somewhat higher than in 2007 (mean of 53.8 m). Mean total outward spread during the course of the 2007 experiment was 29.7 m from the 10 m grid Gourmet treatment plot and 9.8 m from the 20 m grid Gourmet treatment plot. Spread in the two treatment plots was also highest from May through September (Fig. 13). A one-way ANOVA followed by a Tukey HSD test found that total outward spread from the control plot (n = 4 transects) was significantly higher than from the 20 m grid Gourmet plot (t = 4.09, p = 0.007), but was not significantly higher than from the 10 m grid Gourmet plot (t = 1.36, p = 0.402). Mean total spread from the 10 m grid Gourmet plot was marginally significantly higher than from the 20 m grid Gourmet plot (t = 2.73, p = 0.055). It seems unlikely that the lower rate of outward spread from the 20 m grid plot was due to the Gourmet treatment, because the 10 m grid treatment plot had roughly four times as many bait stations yet had a higher rate of outward spread. Instead, it is more likely that the different rates at the different plots mostly reflect natural spatial variation in rates of spread. In any event, neither treatment plot was successful in completely stopping outward spread, which is not surprising given that little ant control was achieved in either plot.

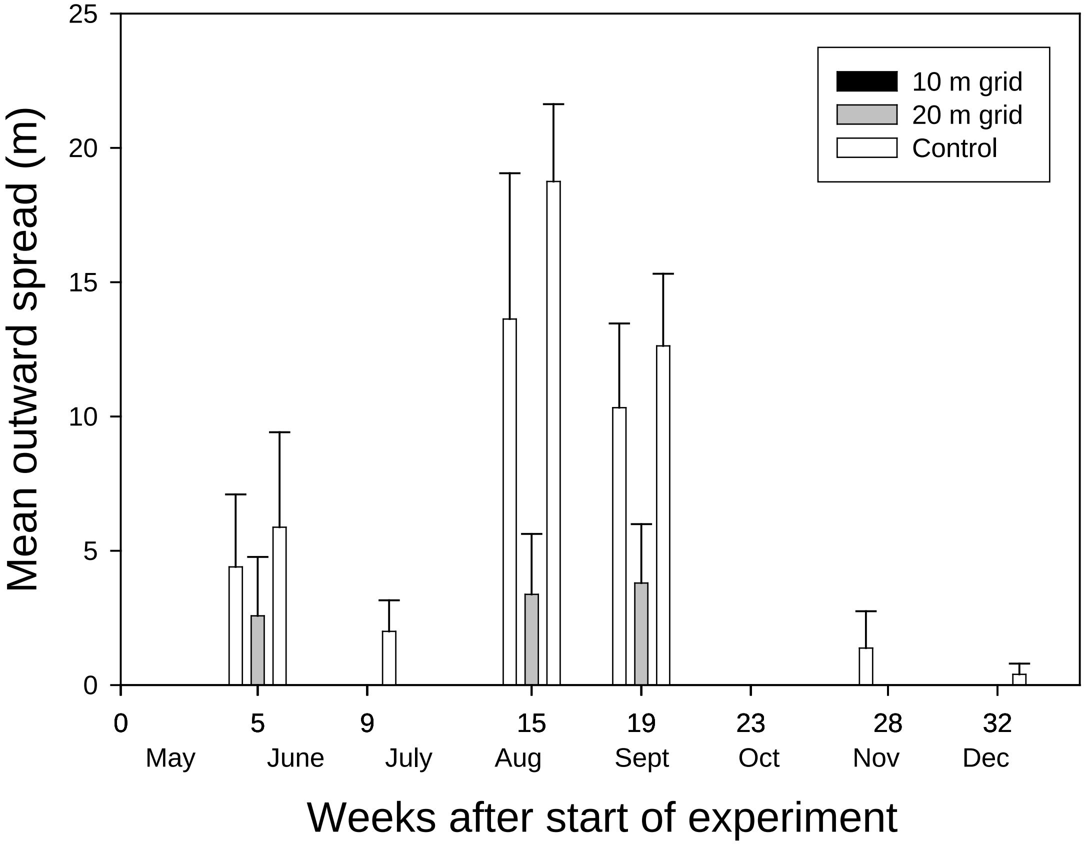

Figure 13. Mean outward spread of the lower ant population at each plot during the interval beginning from the previous monitoring event. For example, spread at week 5 occurred during weeks 0 to 5, and spread at week 9 occurred during weeks 5 to 9. There was no outward spread in any plots during weeks 19 to 23. Spread was measured along 4 parallel transects emanating from each plot. Bars indicate one standard error.

5. CONCLUSIONS

In order for slow-acting borates to be effective, ants must steadily consume, share and distribute relatively large quantities of borate-laced bait across many nests and over fairly long time periods. For example, other studies have found that ants often need to feed on borate baits for 2 to 4 weeks before substantial reductions in worker numbers or activity begins (Klotz et al. 1997b, Greenberg et al. 2006), with reductions sometimes continuing for 10 weeks (Klotz et al. 1998). Sugar water based liquid baits are highly suitable for delivering borate toxicants because they exploit the natural honeydew and nectar gathering behaviors that are so prevalent and important among invasive ants (Lach 2003). As a food carrier, sugar water should therefore allow the borate toxicant to be distributed widely, potentially among all nests and colony members, before it takes effect. In this trial, however, Gourmet Liquid Ant Bait failed to yield substantial control of Argentine ants at HALE, and clearly failed to eradicate the ants from experimental plots, even after being available for 32 weeks.

The explanation for this failure was an insufficient level of bait attractiveness in the experimental plots: ants very rarely recruited high numbers of nestmates to the bait stations, and consumption of the bait was therefore low. The underlying reason for this low level of attractiveness of the Gourmet bait, however, is unclear and a bit puzzling. One possibility is that some ingredient, or a combination of ingredients, in Gourmet Liquid Ant Bait is reducing attractiveness to Argentine ants at HALE. The fact that the preservative used in Gourmet, sodium benzoate, reduced the attractiveness of a 25% sugar water and boric acid solution in a bait preference test at HALE supports this idea. It is possible that the borate toxicant, DOT, also reduces attractiveness somewhat. However, in a previous experiment at HALE, a 25% sugar water + 0.5% boric acid bait formulation was readily consumed by Argentine ants, and another study found that Argentine ants were repelled by borates in sugar water solutions only when their concentrations exceeded 1% (Klotz et al. 2000). Moreover, Gourmet Liquid Ant Bait, and a 50% dilution of Gourmet Liquid Ant Bait, were both found to be attractive and effective in controlling Argentine ants in a California citrus orchard (Greenberg et al. 2006). Nevertheless, the fact that formulations that involved diluting the Gourmet bait were consistently more attractive than undiluted Gourmet in this study suggests that some ingredient or combination of ingredients in the bait reduces attractiveness at HALE. The beneficial effect of dilution is unlikely to be strongly related to bait viscosity, since formulations that diluted Gourmet with 25% sugar water (which should result in minimal changes to total sugar concentration) were also more attractive than undiluted Gourmet bait.

It remains somewhat difficult to explain the large discrepancy between consumption of bait formulations when used in bait preference tests versus in the experimental plots. This type of result has been encountered with at least two other baits at HALE, where performance in bait preference tests did not accurately predict effectiveness in experimental plots (W. Haines and P. Krushelnycky unpublished data). In the present study, both Gourmet formulations that were chosen to be used in the experimental plots exhibited fairly high levels of attractiveness in bait preference tests, but then performed poorly in the plots. As a revealing example, on several occasions during nest surveys in the plots, I found thriving nests within one to two meters of a bait station, with only a few workers inside the station. The discrepancy in attractiveness between bait preference tests and the experimental plots may be related to differences in ant densities in the two areas: the bait preference tests were conducted in a location with high ant densities, while the plots were placed along an ant population boundary where densities tend to be lower. In contrast to many reports from other locations, Argentine ants at HALE rarely tend hemipteran insects for honeydew, despite the fact that this behavior can sporadically be observed and appropriate hemipteran mutualists therefore occur in the park. However, native delphacid planthoppers and introduced aphids are common in the shrubland, and both of these insect groups produce and cast off honeydew (without being actively tended). It is possible that this ambient level of available honeydew is sufficient to meet the carbohydrate needs of Argentine ants at HALE when they occur at medium to low densities, and therefore active tending of hemipterans or recruitment to other sugar sources (like Gourmet) is unnecessary. Perhaps only when ant densities get higher and carbohydrate needs become greater does Gourmet become more attractive. Similar results were reported in a study conducted in a California vineyard, where sugar water boric acid baits yielded much less control when Argentine ant densities were low than when densities were high (Nelson and Daane 2007). In addition, research in California has shown that the proportion of protein versus carbohydrate consumed by Argentine ants varies spatially, with ants at the invasion front consuming more protein and those behind the front consuming more carbohydrate (Tillberg et al. 2007). Although the Gourmet formulations I used contained both carbohydrate and protein, spatial patterns may nevertheless have had some influence on bait attractiveness.

Despite the poor results in this study, sugar water based borate baits have the potential to be effective in other situations in Hawaii, simply because of the well known importance and attractiveness of liquid carbohydrate food sources for invasive ants. Gourmet Liquid Ant Bait, or similar products, may be more attractive to other invasive ants species, or even to Argentine ants in other locations. Basic trials should be conducted to explore these possibilities, and some of the results from this study may be helpful in this regard. For example, the bait station design used in this study could be used in a variety of situations, including attached to tree trunks for species that nest arboreally. In addition, the effectiveness of casein additives should be tested with other ant species. Casein greatly increased attractiveness of sugar water baits for Argentine ants at HALE, and has the potential to do so for other species. Although additives make bait preparation more laborious, casein can be purchased in bulk quantities relatively cheaply. Moreover, protein additives may not only improve bait attractiveness, but may also improve effectiveness in other ways. Baits that include both carbohydrates and protein are less likely to be affected by seasonal fluctuations in colony nutritional needs and food preferences (e.g. Krushelnycky and Reimer 1998a, Rust et al. 2000), and may also be more likely to be shared with all colony castes and life stages, including queens and larvae that require protein for egg production or growth (Markin 1970, Howard and Tschinkel 1981).

ACKNOWLEDGMENTS

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APPENDIX 7. A full report on the evaluation of 0.5 HP Granular Ant Bait is provided in the following pages.

DEVELOPING TECHNIQUES FOR INVASIVE ANT CONTROL: A TEST OF 0.5 HP GRANULAR ANT BAIT ON ARGENTINE ANTS AT HALEAKALA NATIONAL PARK

October 2008

Report to Hawaii Invasive Species Council for PCSU contract 438221

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EXECUTIVE SUMMARY

I investigated the potential of the experimental product 0.5 HP Ant Bait as a tool for eradicating Argentine ants (*Linepithema humile*) in Haleakala National Park (HALE), Hawaii. This experimental bait consists of a mixture of two granule types (a corn grit/soy oil granule and a protein granule) each formulated with a combination of two active ingredients (0.35% hydramethylnon and 0.25% pyriproxyfen). I used three 1 ha treatment plots, plus a fourth 1 ha control plot, to test three bait treatments: corn granules only, protein granules only, and a blend of the two granules. Each treatment received two applications of granules, separated by five to six weeks, at an application rate of 2.24 kg/ha (2 lbs/acre). In addition, excess bait permitted a third application of only the central 30 m by 30 m portions of the corn granule and protein granule treatments (but not the blend treatment). Numbers of ants recruited to baited monitoring cards were strongly reduced after the first and second applications in all three treatments, but never reached 0 in any plots. Incursion of ants into the plots from the periphery was apparent after the first application, but reached less than 25 m into the plots and did not appear to occur after the second application. Nest surveys confirmed the continued presence of active, reproductive nests or nest fragments in the central portions of all three treatment plots throughout the experiment and up to 19 weeks after the initial application. These surviving nests contained eggs and young larvae, suggesting low effectiveness of the insect growth regulator component of the bait (pyriproxyfen), at least under this application protocol. Both baitcard monitoring and nest survey monitoring therefore indicated that eradication did not occur in any of the treated plots, even after two to three applications of 0.5 HP Ant Bait. It was concluded that this product, if used alone, is unlikely to eradicate Argentine ants at HALE, and will likely yield similar results against Argentine ants in other natural area situations in Hawaii. It may, however, be a useful tool in combination with other effective products. Moreover, it produced results comparable to those observed with other bait products formulated with hydramethylnon at concentrations two to three times higher. In this respect, it could become a preferred product for species known to be effectively controlled with hydramethylnon, such as the big-headed ant and (in some situations) the little fire ant.

INTRODUCTION

Invasive ants are among the most damaging of Hawaii's invasive species. There are believed to be no native ants in Hawaii, yet in the past several hundred years over 50 ant species have been introduced to the state. Some of these species have caused substantial impacts to native Hawaiian biodiversity, and are pests of agriculture and urban areas (Krushelnycky et al. 2005). In addition, recent and potential introductions, such as the little fire ant and red imported fire ant, respectively, have the ability to exert strong impacts on tourism and other sectors of the economy (Gutrich et al. 2007).

Techniques for controlling and even eradicating existing populations of the state's most invasive ant species are critical for rapid response to incipient incursions, as well as situations in which biodiversity and other interests can be protected by removing well-established but localized ant populations. Some of the most damaging invasive ant species exhibit a unicolonial social structure in which mating flights do not occur, new queens bud from existing nests and disperse short distances by walking, and populations can therefore exist as discrete, localized entities even when the species' total distribution is much wider. Successful eradication of local populations can thus result in the permanent removal of these species from particular areas of concern, as long as re-introduction by humans can be prevented or quickly detected (Krushelnycky et al. 2005, Silverman and Brightwell 2008).

Efforts to control or eradicate invasive ant populations typically involve the use of attractive baits formulated with insecticidal toxicants. However, different ant species respond to different baits, and different situations call for different active ingredients and methods of application. Developing multiple management tools for invasive ants will greatly improve the state's ability to address these problem species.

At Haleakala National Park (HALE), the Argentine ant (*Linepithema humile*) has emerged as one of the most important threats to endemic subalpine shrubland and alpine zone arthropods. Since at least 1967, the Argentine ant has been slowly but steadily spreading within the park, with two discrete populations now covering over 625 ha. Numerous experiments testing a variety of commercial and experimental pesticidal ant baits have been conducted over the past ten years at HALE in an attempt to develop a method for eradicating the Argentine ant (e.g. Krushelnycky and Reimer 1998a,b). Most of these baits have been granular in form, which are the easiest, cheapest and most practical type of ant bait to use in difficult outdoor applications. While some of the baits tested have been very effective in reducing numbers of ants, none has been able to eliminate all nests in experimental plots. Consequently, no ant bait product tested to date appears to be effective enough to successfully eradicate the two Argentine ant populations in the park. Continued research with additional products is therefore needed to address resource management goals specific to HALE, but also to improve capacity to manage invasive ants in Hawaii in general. In the present study, I evaluated whether the experimental product 0.5 HP Ant Bait has the potential to eradicate Argentine ants at HALE. The results obtained should also have direct relevance to other situations across the state.

0.5 HP Ant Bait is a granular bait currently under development by Sumitomo Chemical Australia. Like several currently available fire ant baits, it is formulated with two active ingredients – a combination of 0.35% hydramethylnon and 0.25% pyriproxyfen. Hydramethylnon is a metabolic inhibitor, and is the same toxicant used in Maxforce Granular Insect Bait, a product tested extensively against Argentine ants at HALE. Pyriproxyfen is an insect growth regulator aimed at halting development of immature stages and sterilizing queens.

This combination of hydramethylnon with an insect growth regulator has been employed in the campaign to eradicate red imported fire ants (*Solenopsis invicta*) in Australia. In addition to using two active ingredients, 0.5 HP Ant Bait is unique in that it combines two different bait carriers – a protein granule and a corn grit granule soaked in soybean oil. Each of these granule types has been commonly used separately in individual ant bait products, but 0.5 HP Ant Bait blends the two types together in a single bait with the goal of improving attractiveness and consumption for a wider variety of pest ant species. The protein granule is composed of fish meal, and has been used as the bait carrier (with a different active ingredient) in yellow crazy ant (*Anoplolepis gracilipes*) control work on Christmas Island. The corn grit/soybean oil granule is similar to that used in a wide variety of baits targeting fire ants, but has been augmented with a proprietary ingredient to improve attractiveness to species, like the Argentine ant, that typically aren't strongly attracted to corn grit/soybean oil based baits. The combination of both granule types may increase effectiveness if bait preferences vary among nests either spatially or temporally (if multiple applications are made). In this experiment, I tested both granule types separately and blended together as in the intended commercial product.

METHODS

I established four 1 ha (100 m by 100 m) experimental plots within the lower Argentine ant population in HALE, in native shrubland between 2225 and 2375 m elevation. The area selected supported high densities of ants prior to the experiment. Three of the plots were randomly assigned to one of three treatments using 0.5 HP Ant Bait: protein granules only, corn granules only, or a 50:50 blend (by weight) of both granules. The fourth plot served as a control and was not treated. Bait was broadcast throughout each of the three treated plots, using handheld "whirlybird" bait spreaders, at an application rate of 2.24 kg/ha (2 lbs/acre) on two occasions: first on 8/21/07-8/22/07, and again on 9/28/07-9/30/07. The second application, roughly 5 to 6 weeks after the first, was intended to target nests and nest fragments that survived the first application, especially individuals that were in the egg or pupal stages at the time of the first application. A small amount of bait remained after the second application, and this was used to treat only the central 30 m by 30 m portions of the protein treatment plot and corn treatment plot (but not the blend treatment plot), at 2.24 kg/ha, on a third occasion on 11/1/07.

I conducted two types of monitoring to assess the efficacy of the treatments: bait card monitoring (using non-toxic attractants) to assess relative ant abundance levels in the plots, and nest surveys to assess survival of queens and immature stages. Bait cards were placed at 40 monitoring stations within each plot, including 12 'outer stations' (12.5 m from the plot border), 12 'middle stations' (25 m from the plot border), 12 'inner stations' (35 m from the plot border), and 4 'central stations' (45 m from the plot border) (Fig. 1). During each monitoring event, I provisioned each bait card with about 1.5 g of a blend of 40% tuna (in water) and 60% light corn syrup, by weight, and placed the bait card on the ground and in the shade for a period of 60 minutes. At the end of 60 minutes, I counted the number of ants on each card. Bait card monitoring was conducted on 8/18/07-8/19/07 (pre-treatment), and approximately every week after the initial ant bait application until mid-November 2007 (84 days post-treatment), as well as on two additional occasions 98 and 136 days after the initial application.

Nest survey monitoring was conducted in the central 50 m by 50 m portions of each plot, which were divided into 25 10 m by 10 m quadrats (Fig. 1). For each nest survey, I randomly selected one of the nine central 10 m by 10 m quadrats for monitoring, with the exception of the pre-treatment survey quadrat, which was randomly selected from the 16 outer quadrats. All post-treatment monitoring quadrats were therefore located at least 35 m from the edge of the plot, and the quadrat in the direct center of the plot was reserved for the final monitoring event because it occurred at the longest time interval after treatment. During each monitoring event, every rock in the selected quadrat was overturned in search of nests. All nests within the quadrat were marked and recorded as reproductive (presence of queens, eggs, larvae or pupae) or nonreproductive (presence only of workers or males; or evidence of prior use as a nest site, such as presence of nest galleries). Numbers of individuals in each caste/stage were recorded, according to abundance categories: 0 = 0, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 101-500, 5 = >500. Nest surveys were conducted between 9:00 am and 12:00 pm on warm sunny days, when ants bring brood up to the soil surface (underneath the cover rock) presumably to take advantage of warmer temperatures. Surveys were occasionally conducted slightly later on cooler days. Nest surveys were conducted on 8/18/07-8/19/07 (pre-treatment), and at roughly 4, 7, 8, 9, 12, 14 and 19 weeks after the initial ant bait application.

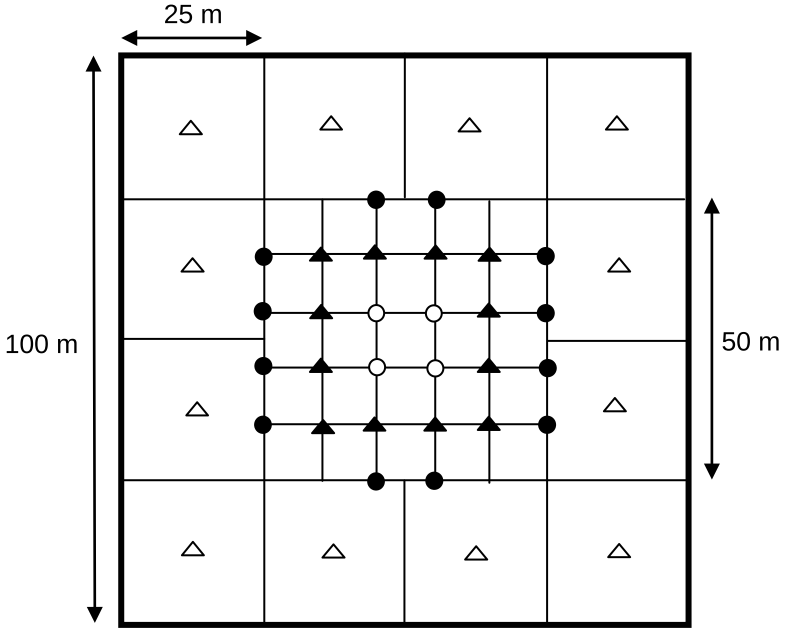

Figure 1. Layout of the plots. The central 50 m by 50 m portions of the plots were divided into 25 10 m by 10 m quadrats for the nest surveys. Symbols indicate the locations of bait card monitoring stations, as follows: empty triangles = outer stations, filled circles = middle stations, filled triangles = inner stations, empty circles = central stations.

On 11/1/07 and 11/8/07 I conducted an open choice bait preference test between the two types of granules (corn and protein) used in 0.5 HP Ant Bait. I conducted four replicate choice tests on each of the two dates. In each test, two index cards were placed side by side on the ground, and a small pile of one of the two baits was placed on each card. On the first date

(11/1/07), 2 g of granules were used for each index card, while 1 g of granules was used for each index card on the second date (11/8/07). The bait preference test was conducted in a high ant density area near the four experimental plots, and the 8 replicate choice tests were located in shaded spots that were separated from each other by at least 5 m. After placing the baits on the cards, I counted numbers of ants on each bait at 5 minute intervals for the first 30 minutes, and then every 10 minutes for the following hour (up to 90 minutes total length).

RESULTS

The first application of all three bait treatments (corn, protein, and blend) strongly reduced the number of ants recruited to monitoring bait cards (Fig. 2). In none of the plots, however, were numbers reduced to zero. Ant numbers at bait card stations recovered by 16.6% to 45.7% in the treated plots from three to five weeks after the first application. This recovery appeared to be strongest in the corn granule plot, weakest in the protein granule plot, and intermediate in the blend plot (Fig. 2). However, because there was only one plot per treatment type, and because the corn plot had the highest numbers of ants prior to treatment, it is difficult to judge the probability of whether this pattern actually indicates a stronger suppressive effect of the protein granules relative to the corn granules.

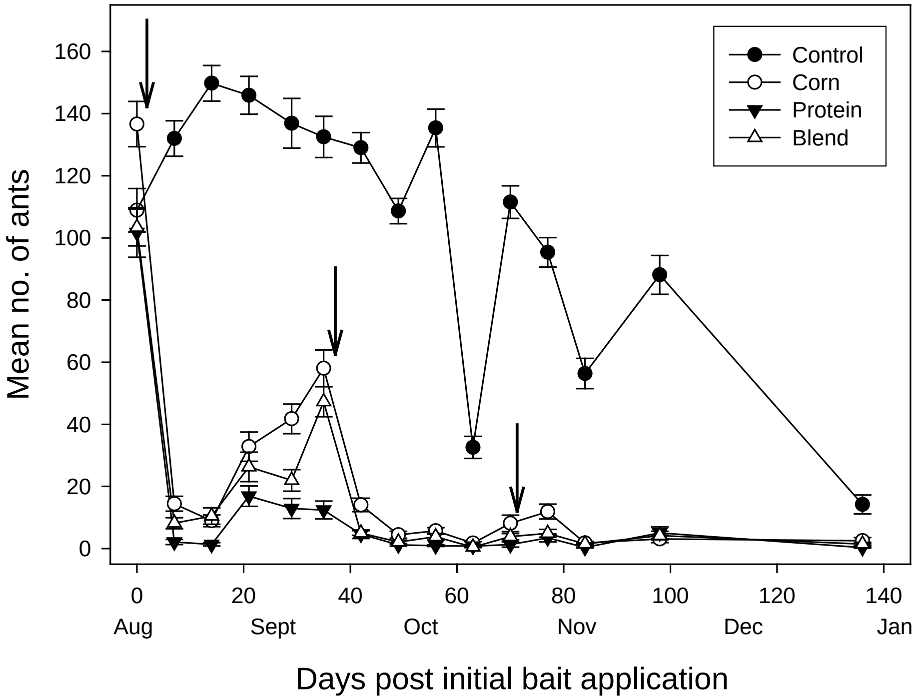

Figure 2. Mean numbers of ants (±1 SE) at monitoring bait cards (n=40) in the four plots during the course of the experiment. Arrows indicate timing of pesticidal ant bait applications. The first two applications were made in all three treatment plots, while the third application was only made in the central portions of the corn and protein plots (see Methods).

It is clear that some of the recovery in ant numbers at bait cards was due to foraging or re-colonization from the plot borders. In all three treatment plots, ant numbers at the outer bait card stations became substantially higher than those at the middle or inner stations at two to three weeks after the application (Fig. 3). This pattern did not occur in the control plot, where ant numbers at the three bait card station types were similar throughout the experiment, with no consistent bias towards higher numbers near the plot periphery. This re-invasion appeared to extend less than 25 m into the treatment plots by 5 weeks after the application (since middle stations did not have higher numbers of ants than inner stations), strongly suggesting that the monitoring results in the central 50 m by 50 m portions of the plots reflected the true treatment effects.

Nest surveys at 4 weeks after the first application confirmed that a substantial number of nests or nest fragments survived in the central portions of all three treatment plots (Figs. 4-7). Nest survey data need to be interpreted with caution due to the high natural spatial variability in nest density. Because a different quadrat was surveyed during each monitoring event, differences between monitoring events in densities of nests or abundances of particular castes or life stages potentially represent natural spatial differences as much as or more than they represent temporal trends in these metrics. Elucidating subtle temporal and/or treatment-induced effects in the nest survey data is therefore difficult. However, nest surveys are highly effective for confirming or discounting a dramatic result, such as eradication, that might be suggested from bait card monitoring. After the first application, queens, eggs, worker larvae, and hence reproductive nests, were all present and fairly abundant in the three treated plots, clearly indicating that eradication had not occurred.

The second broadcast application of the granular baits once again strongly reduced the numbers of ants recruited to the monitoring bait cards in all three treated plots (Fig. 2). Again, numbers of ants at bait cards did not drop to zero in any of the plots. Unlike the first application, there was no obvious recovery in ant numbers in the four to five weeks after the second application in any of the treated plots, indicating that two applications of the baits had a greater suppressive effect on worker numbers than a single application. It also suggests that the corn and protein granules (as well as the blend of the two) are similar in their effectiveness when more than one application is made.

There was much less re-colonization from outside the treated plots after the second application, with weak evidence of this phenomenon apparent only in the corn plot (Fig. 3). It was therefore clear that persisting forager ants in all three treated plots came from surviving nests or nest fragments. Queens, eggs, worker larvae and reproductive nests were all present at two to four weeks after the second application (Figs. 4-7).

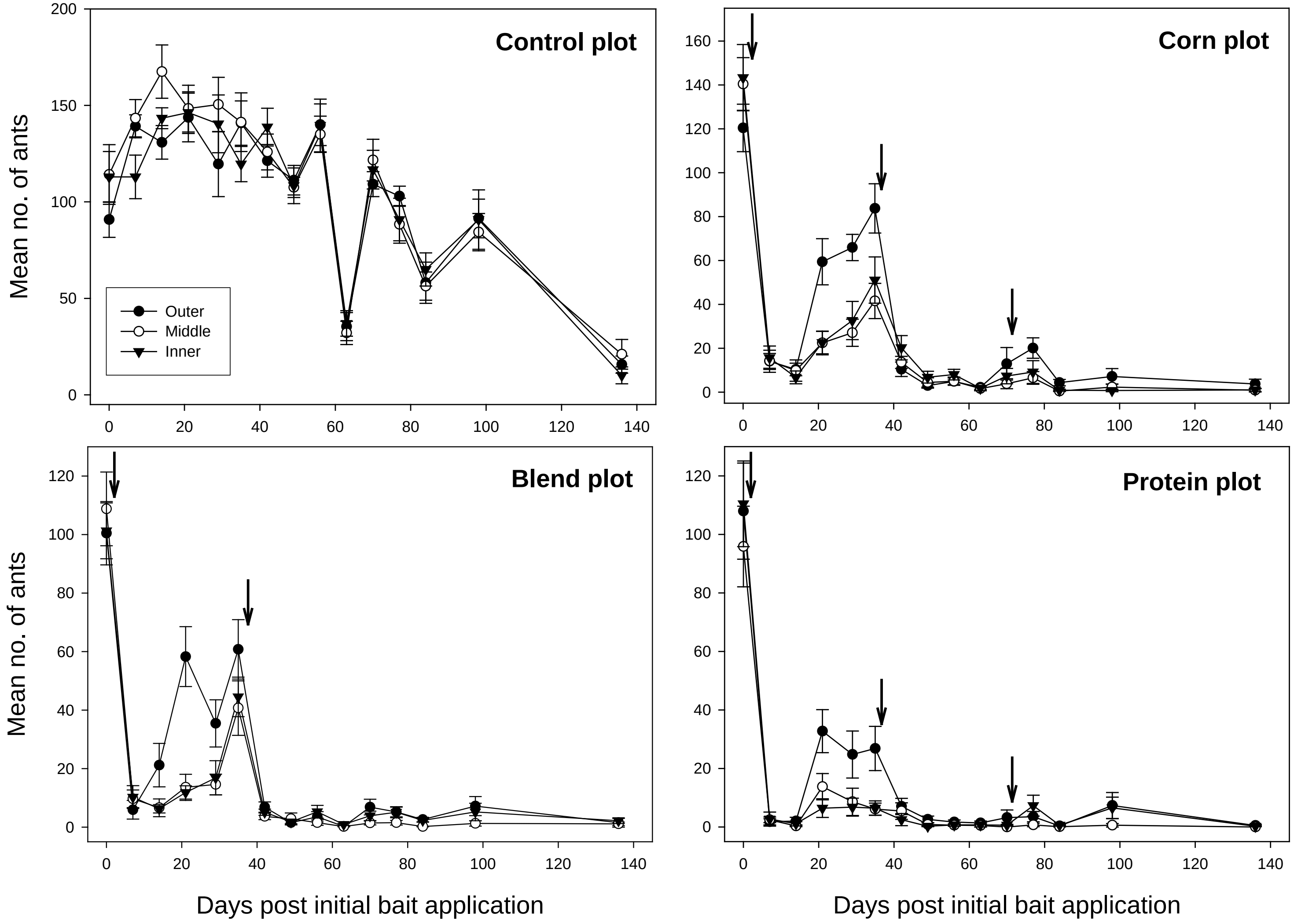

Figure 3. Mean numbers of ants (±1 SE) at three of the four bait card monitoring station types in each of the four plots during the course of the experiment. Arrows indicate timing of pesticidal ant bait applications. See Methods and Figure 1 for the relative positions of the three monitoring station types within the plots.

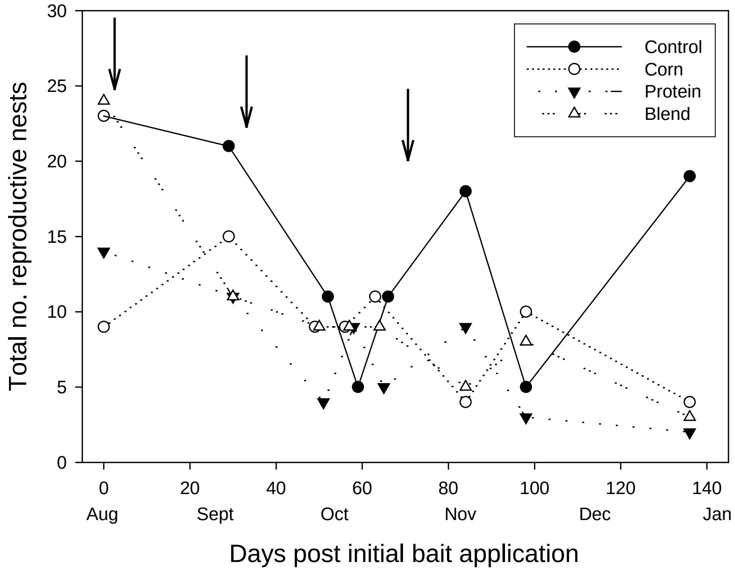

Figure 4. Total number of reproductive nests per monitoring quadrat in the four plots during the course of the experiment. Arrows as in Fig. 2.

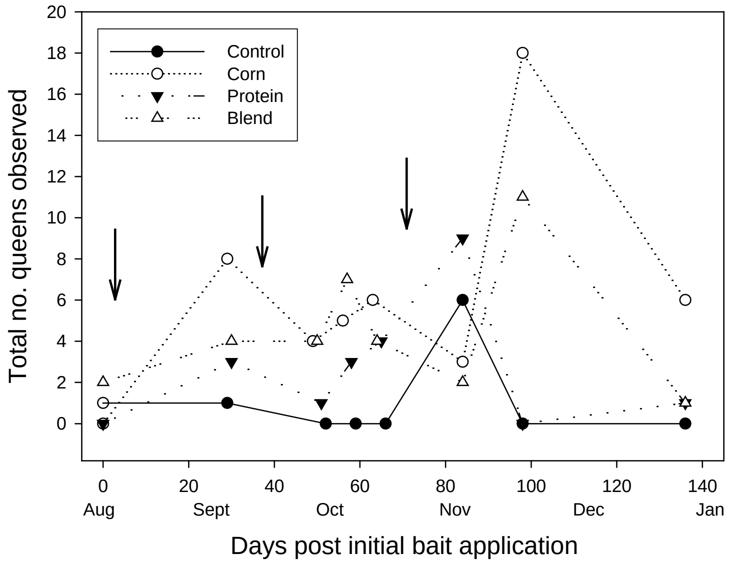

Figure 5. Total number of queens observed per monitoring quadrat in nest surveys in the four plots during the course of the experiment. Arrows as in Fig. 2.

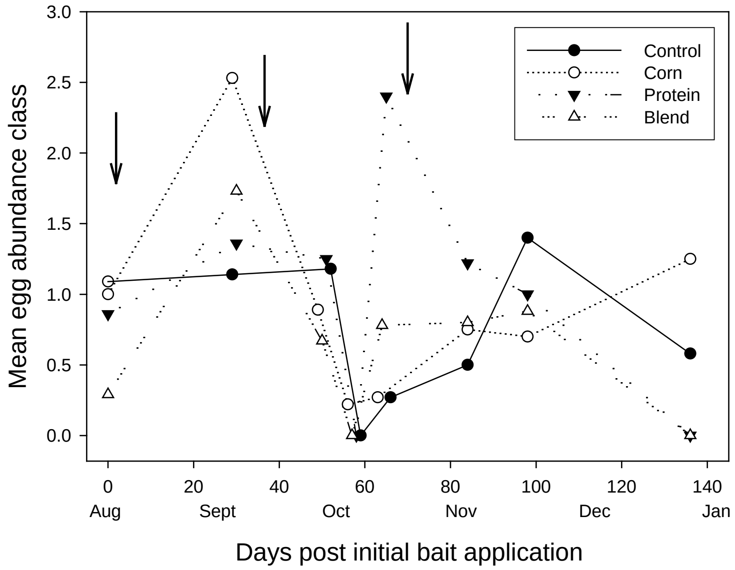

Figure 6. Mean egg abundance class of reproductive nests during nest surveys in the four plots during the course of the experiment. Arrows as in Fig. 2.

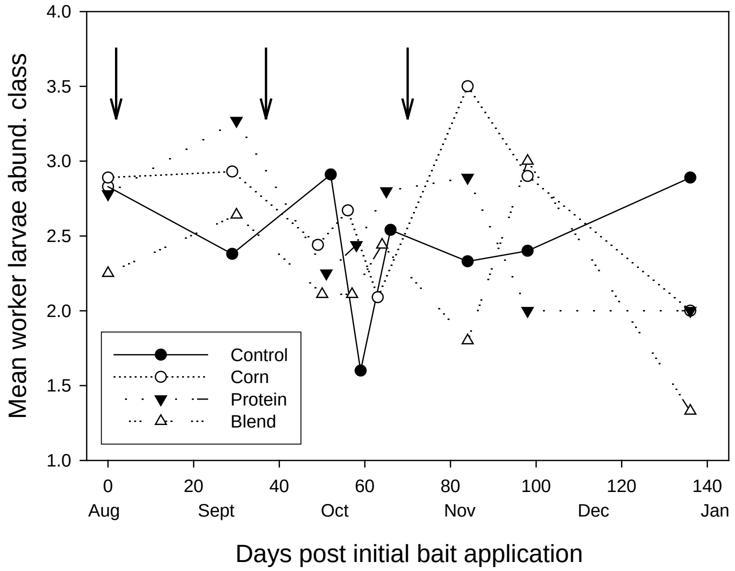

Figure 7. Mean worker larvae abundance class of reproductive nests during nest surveys in the four plots during the course of the experiment. Arrows as in Fig. 2.

In a last attempt at eradication using 0.5 HP Ant Bait, I used the remaining corn and protein granules in a third application of the central 30 m by 30 m portions of the corn and protein plots only, approximately five weeks after the second application. Ant numbers at bait cards were still relatively low from the second application, and dropped again one to two weeks after the third application, but did not reach zero in any of the plots (Figs. 2 and 3). Bait card numbers did not recover substantially over the remainder of the experiment in the three treatment plots (even at roughly nine weeks after the third application), however this was probably due in large part to the fact that ant population levels were naturally dropping sharply as part of a regular seasonal decline (see control plot in Figs. 2 and 3, Krushelnycky et al. 2004). Despite the low numbers of ants recruited to monitoring bait cards, nest surveys conducted at two, four and nine weeks after the third application once again clearly indicated that eradication was not achieved in the experimental plots (Figs. 4-7).

The number of ants attracted to the 0.5 HP Ant Bait during the bait preference test was surprisingly low but consistently higher for the corn granules as compared to the protein granules (Figs. 8 and 9). A paired t-test conducted on the mean counts of the eight replicate trials found that the number of ants attracted to the corn granules was significantly higher than the number attracted to protein granules (t = 3.19, p = 0.015).

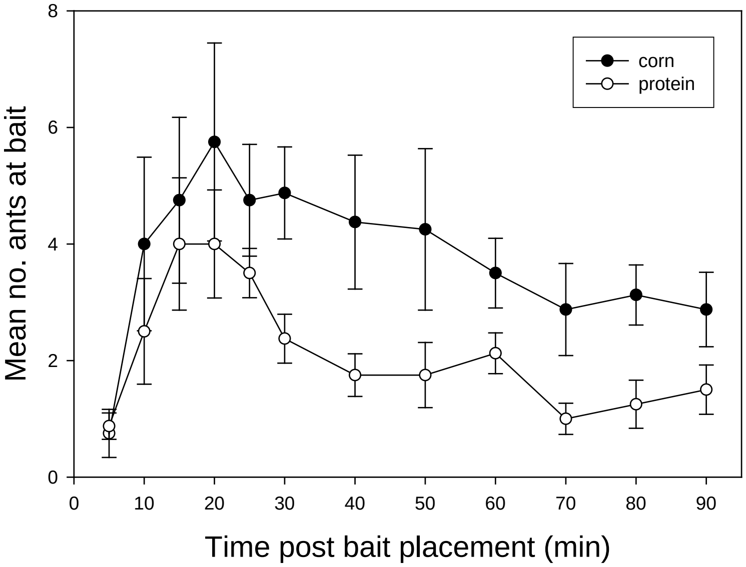

Figure 8. Numbers of ants attracted to the two granule types in 0.5 HP Ant Bait over the course of 90 minutes in side-by-side bait preference trials. Data shown are the means (± 1 SE) of eight replicate trials.

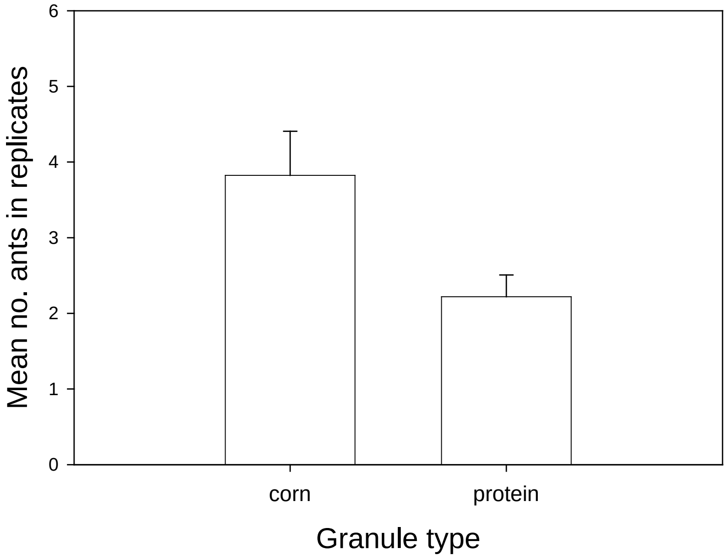

Figure 9. Numbers of ants attracted to the two granule types in 0.5 HP Ant Bait in side-by-side bait preference trials. Data shown are the means (± 1 SE) of the average counts of the eight replicate trials.

DISCUSSION

This experiment found that 0.5 HP Ant Bait is unlikely to eradicate Argentine ants at HALE with two or three broadcast applications. Reproductive nests or nest fragments always survived in the centers of the 1 ha plots, and this was true for each of the two granule types in the bait as well as for the two types blended together (as intended in the commercial product). One of the two active ingredients in 0.5 HP Ant Bait, hydramethylnon, typically induces mortality within several days to a week (Reimer and Beardsley 1990, Krushelnycky and Reimer 1998b), so this 19 week-long experiment was more than sufficient for concluding that eradication did not result from exposure to this toxicant. The second active ingredient, the insect growth regulator pyriproxyfen, acts more slowly on adult populations because these are mainly affected when interruption of egg production and development of immatures begins to prevent the replacement of senescing workers. This may take several weeks to 12 weeks or more (Reimer et al. 1991, Vail et al. 1996, Souza et al. 2008). However, impacts on reproductive output should be manifest within several weeks (Reimer et al. 1991, Vail et al. 1996), and the fact that eggs and young larvae were still present in the nests of treated plots, and often comparably abundant to those in the control plot, at 14 to 19 weeks after the initial application suggests that the pyriproxyfen had only minimal effects. In fact, the results of the 0.5 HP Ant Bait were very similar to those obtained in previous trials with Maxforce Granular Insect Bait (e.g. Krushelnycky and Reimer 1998b, Krushelnycky et al. 2004), which uses only hydramethylnon as an active ingredient. This may be because the relatively rapid toxic effects of hydramethylnon interfere with the efficacy of the growth regulator, or perhaps a more continuous exposure to pyriproxyfen is necessary to yield significant reproductive control under field conditions (Souza et al. 2008).

Although the two granule types in 0.5 HP Ant Bait yielded similar control of worker ants attracted to bait cards after multiple applications, there was some evidence that after only one application the protein granule resulted in greater ant suppression than the corn granule (with the blend of the two being intermediate). This was surprising because Argentine ants at HALE prefered the corn granule over the protein granule in a side-by-side bait preference test. The formulation of the Sumitomo Chemical Australia corn granule therefore does appear to be more attractive to Argentine ants than traditional corn granule-based baits (e.g. Amdro), and even more attractive than the fish-based protein granule in 0.5 HP Ant Bait, at least when it is initially encountered by foraging ants. For some reason, however, this initial attractiveness does not translate into greater efficacy when broadcast, and in fact may yield lower efficacy than the less attractive protein granule. One potential explanation may be that while an effective attractant may induce workers to pick up the corn granules preferentially, nutritional needs may dictate that the protein granules are preferentially consumed once back in the nest. More generally, a disconnect between results from bait preference tests and field applications has been encountered in prior studies at HALE. In one example, another corn grit/soybean oil-based granular bait (Advance Granular Carpenter Ant Bait) that was extremely attractive in a bait preference test yielded little control when applied in experimental plots (W. Haines unpubl. data). In a second example, several formulations of Gourmet Liquid Ant Bait were found to be quite attractive in bait preference tests but were then largely ignored when placed in bait stations in experimental plots (P. Krushelnycky unpubl. data). This recurring theme strongly suggests that while bait preference tests may provide some useful information, field experiments must ultimately be performed to accurately assess the efficacy of ant bait products.

It seems likely that similar results will be obtained when using 0.5 HP Ant Bait, under the same application protocol, against Argentine ants in other situations in Hawaii. It could, however, become a highly useful tool in combination with other granular products under development, particularly if a suite of products formulated with different bait carriers and/or toxicants were to be used. Furthermore, the results in this study suggest that baits formulated with only 0.35% hydramethylnon can yield levels of control similar to baits formulated with 0.7% to 1.0% hydramethylnon (e.g. Amdro, Maxforce GIB). Because of its lower concentration of active ingredient, 0.5 HP Ant Bait, if eventually available for non-experimental use in Hawaii, may become a preferred product for use on species that are known to be effectively controlled with hydramethylnon (e.g. the big-headed ant, *Pheidole megacephala*, Reimer and Beardsley 1990, Hoffmann and O'Connor 2004; and in some situations the little fire ant, *Wasmannia auropunctata*, Abedrabbo 1994, Causton et al. 2005).

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APPENDIX 8. A full report on the evaluation of Advion Insect Granule bait is provided in the following pages.

INVASIVE ANT CONTROL FOR NATIVE ECOSYSTEM PRESERVATION AND RESTORATION IN HAWAII: A TEST OF ADVION INSECT GRANULE BAIT ON ARGENTINE ANTS AT HALEAKALA NATIONAL PARK

January 2010

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EXECUTIVE SUMMARY

Advion Insect Granule (IG), a relatively new granular bait product formulated with the active ingredient indoxacarb, was tested as a potential tool for eradicating or controlling Argentine ants in Haleakala National Park (HALE). Advion IG performed differently at different scales and in different settings. Advion IG caused high mortality of worker ants (>90%) in laboratory colonies in 10 days, although only 33% of queens died in this time period. In comparison, less than 15% of workers died in laboratory colonies offered Maxforce Granular Insect Bait (GIB) in 10 days, and no queens died. Maxforce GIB has been used on numerous occasions in attempts to eradicate or slow spread of Argentine ants at HALE, typically causes high mortality of worker ants, and therefore serves as a useful standard with which to compare new bait products. The low mortality of workers in laboratory colonies offered Maxforce GIB may have been due in part to higher attractiveness of other protein and carbohydrate food sources that were provided throughout the experiment.

In a field-based bait preference test, Advion IG granules were significantly less attractive than Maxforce GIB granules, with roughly four times as many ants attracted to the Maxforce GIB. The relatively low attractiveness of Advion IG was consistent with poor results in 1 ha experimental efficacy plots. In two separate plots, a single broadcast application of Advion IG at 2.24 kg/ha (2 lbs/acre) yielded no reduction in numbers of workers attracted to monitoring bait cards. Lower numbers of ants at four and five weeks after application were observed in both treated plots as well as the control plot, suggesting that this fluctuation was due to external factors such as weather. Numerous active, reproductive nests persisted in both treated plots. A second application of Advion IG five weeks after the first in one of the plots also yielded no discernable reduction in numbers of worker ants in the four weeks following treatment. In the other treated plot, a broadcast application of Maxforce GIB, at 1.68 kg/ha (1.5 lbs/acre, the label rate), five weeks after the initial Advion IG application resulted in a substantial reduction in numbers of workers at monitoring bait cards, similar to results obtained in prior tests with Maxforce GIB. Multiple reproductively active nests persisted in both treated plots, however. These results strongly suggest that Advion IG, either alone or in combination with another bait product, is unlikely to yield effective control of Argentine ants in natural area settings in Hawaii, at least under the application protocol tested.

Previous trials of ant control products at HALE have often found that bait preference tests do not accurately predict outcomes in field trials. This was not true in the present study, however tests of Advion IG at different scales yielded completely different results. It is hypothesized that the much higher mortality of workers in laboratory colonies resulted from topical exposure incurred while investigating and contacting the granules and subsequent self-cleaning. The level of incidental exposure of worker ants to granules broadcast in the field is undoubtedly dramatically lower, and may explain the lack of observed mortality in the field plots.

INTRODUCTION

Invasive ants are among the most damaging of Hawaii's invasive species. There are believed to be no native ants in Hawaii, yet in the past several hundred years over 50 ant species have been introduced to the state. Some of these species have caused substantial impacts to native Hawaiian biodiversity, and are pests of agriculture and urban areas (Krushelnycky et al. 2005b). In addition, recent and potential introductions, such as the little fire ant and red imported fire ant, respectively, have the ability to exert strong impacts on tourism and other sectors of the economy (Gutrich et al. 2007).

Techniques for controlling and even eradicating existing populations of the state's most invasive ant species are critical for rapid response to incipient incursions, as well as situations in which biodiversity and other interests can be protected by removing well-established but localized ant populations. Some of the most damaging invasive ant species exhibit a unicolonial social structure in which mating flights do not occur, new queens bud from existing nests and disperse short distances by walking, and populations can therefore exist as discrete, localized entities even when the species' total distribution is much wider. Successful eradication of local populations can thus result in the permanent removal of these species from particular areas of concern, as long as re-introduction by humans can be prevented or quickly detected (Krushelnycky et al. 2005b, Silverman and Brightwell 2008).

Efforts to control or eradicate invasive ant populations typically involve the use of attractive baits formulated with insecticidal toxicants. However, different ant species respond to different baits, and different situations call for different active ingredients and methods of application. Developing multiple management tools for invasive ants will greatly improve the state's ability to address these problem species.

At Haleakala National Park (HALE), the Argentine ant (*Linepithema humile* [Mayr]) has emerged as one of the most important threats to endemic subalpine shrubland and alpine zone arthropods. Since at least 1967, the Argentine ant has been slowly but steadily spreading within the park, with two discrete populations now covering over 625 ha. Numerous experiments testing a variety of commercial and experimental pesticidal ant baits have been conducted over the past ten years at HALE in an attempt to develop a method for eradicating the Argentine ant (e.g. Krushelnycky and Reimer 1998a,b). Most of these baits have been granular in form, which are the easiest, cheapest and most practical type of ant bait to use in difficult outdoor applications. While some of the baits tested have been very effective in reducing numbers of ants, none has been able to eliminate all nests in experimental plots. Consequently, no ant bait product tested to date appears to be effective enough to successfully eradicate the two Argentine ant populations in the park. Continued research with additional products is therefore needed to address resource management goals specific to HALE, but also to improve capacity to manage invasive ants in Hawaii in general. In the present study, I evaluated whether the product Advion Insect Granule (IG) has the potential to eradicate Argentine ants at HALE. The results obtained should also have direct relevance to other situations across the state.

Advion IG is a granular bait produced by DuPont, and consists of a proprietary bait carrier formulated with 0.22% of the active ingredient indoxacarb. Indoxacarb is a relatively new, fast-acting nerve toxicant, and has been found to be effective in controlling fire ants when formulated in DuPont's Advion Fire Ant Bait. Like other fire ant baits, Advion Fire Ant Bait uses a corn grit and soybean oil granular bait carrier, which unfortunately is not attractive to Argentine ants. Advion IG is currently the only available granular bait formulated with indoxacarb that uses a bait carrier different from the standard corn grit/soybean oil carrier. While Advion IG was originally targeted for mole cricket control (it was previously marketed under the name Advion Mole Cricket Bait), DuPont reports that it has also shown good efficacy against several pest ant species, including Argentine ants in laboratory trials (M. Coffelt, pers. comm.). Here, I tested two different treatment types using Advion MCB against Argentine ants in experimental field plots at HALE. The first treatment consisted of two sequential applications of Advion IG at a rate of 2.24 kg/ha (2 lbs/acre). While the Advion IG label allows application rates of up to 200 lbs/acre in turfgrass, 2 lbs/acre is similar to application rates used for other granular ant baits at HALE, and is more in line with the label application rate of Advion Fire Ant Bait (1.5 lbs/acre). The two applications would be separated by about five to six weeks, with the second application intended to target nests and nest fragments that survived the first application, especially individuals that were in the egg or pupal stages at the time of the first application. The second treatment type consisted of one application of Advion IG at a rate of 2.24 kg/ha, followed five to six weeks later by an application of Maxforce Granular Insect Bait (GIB) at a rate of 1.68 kg/ha (1.5 lbs/acre, label rate). Maxforce GIB (formulated with 1.0% hydramethylnon) has been the most commonly used ant bait in efforts to control Argentine ants at HALE. It is fairly attractive to Argentine ants and typically results in high levels of worker ant reduction, although nest fragments always survive. The second treatment type in this experiment was designed to test whether the sequential use of two different baits (including different bait carriers and active ingredients) may increase effectiveness. For example, bait preferences could vary among nests either spatially or temporally, or surviving nests could become “bait shy” and avoid a particular bait after the initial exposure.

In combination with this field test of Advion IG efficacy, I investigated the attractiveness of Advion IG in the field (relative to Maxforce GIB), and the efficacy of Advion IG for controlling laboratory Argentine ant colonies (also compared to Maxforce GIB). These additional tests would reveal how Advion IG performs at different scales and in different settings, and indicate whether certain tests may reliably predict outcomes of large-scale control efforts.

METHODS

Field efficacy plots

Field testing of Advion IG at HALE involves a use pattern that is not covered by the specific label language of the product. An Experimental Use Permit, issued by the Hawaii Department of Agriculture, was therefore necessary to conduct the experiment. In addition, Section 7 consultation with the US Fish and Wildlife Service was deemed necessary because of potential impacts on endangered nene (Hawaiian goose, *Branta sandvicensis*) that occasionally occur in the proposed study area. I obtained both forms of regulatory approval prior to the initiation of the experiment.

I established three 1 ha (100 m by 100 m) experimental plots within the lower Argentine ant population in HALE, in native shrubland between 2225 and 2375 m elevation. The area selected supported high densities of ants prior to the experiment. Two of the plots were randomly assigned to one of two treatments: two successive applications of Advion IG at 2.24 kg/ha (2 lbs/acre), or a single application of Advion IG at 2.24 kg/ha followed by a single application of Maxforce GIB at 1.68 kg/ha (1.5 lbs/acre). Bait was broadcast throughout each of the two treated plots using handheld “whirlybird” bait spreaders, first on 8/13/08 and again on 9/17/08, five weeks later. The third plot served as a control and was not treated.

I conducted two types of monitoring to assess the efficacy of the treatments: bait card monitoring (using non-toxic attractants) to assess relative ant abundance levels in the plots, and nest surveys to assess survival of queens and immature stages. Bait cards were placed at 40 monitoring stations within each plot, including 12 ‘outer stations’ (12.5 m from the plot border), 12 ‘middle stations’ (25 m from the plot border), 12 ‘inner stations’ (35 m from the plot border), and 4 ‘central stations’ (45 m from the plot border) (Fig. 1). During each monitoring event, I provisioned each bait card with about 1.5 g of a blend of 40% tuna (in water) and 60% light corn syrup, by weight, and placed the bait card on the ground and in the shade for a period of 60 minutes. At the end of 60 minutes, I counted the number of ants on each card. Bait card monitoring was conducted on 8/7-8/8/08 (pre-treatment), and approximately every 7 days after the initial ant bait application until 10/21-10/22/08 (70 days post-treatment).

Nest survey monitoring was conducted in the central 50 m by 50 m portions of each plot, which were divided into 25 10 m by 10 m quadrats (Fig. 1). For each nest survey, I randomly selected one of the nine central 10 m by 10 m quadrats for monitoring, with the exception of the pre-treatment survey quadrat, which was randomly selected from the 16 outer quadrats. All post-treatment monitoring quadrats were therefore located at least 35 m from the edge of the plot, and the quadrat in the direct center of the plot was reserved for the final monitoring event because it occurred at the longest time interval after treatment. During each monitoring event, every rock in the selected quadrat was overturned in search of nests. All nests within the quadrat were marked and recorded as reproductive (presence of queens, eggs, larvae or pupae) or nonreproductive (presence only of workers or males; or evidence of prior use as a nest site, such as presence of nest galleries). Numbers of individuals in each caste/stage were recorded, according to abundance categories: 0 = 0, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 101-500, 5 = >500. Nest surveys were conducted between 9:00 am and 12:00 pm on warm sunny days, when ants bring brood up to the soil surface (underneath the cover rock) presumably to take advantage of warmer temperatures. Surveys were occasionally conducted slightly later on cooler days. Nest surveys were conducted on 8/7-8/8/08 (pre-treatment); at 2 and 4 weeks after the first bait application; and at 1, 3 and 5 weeks after the second bait application.

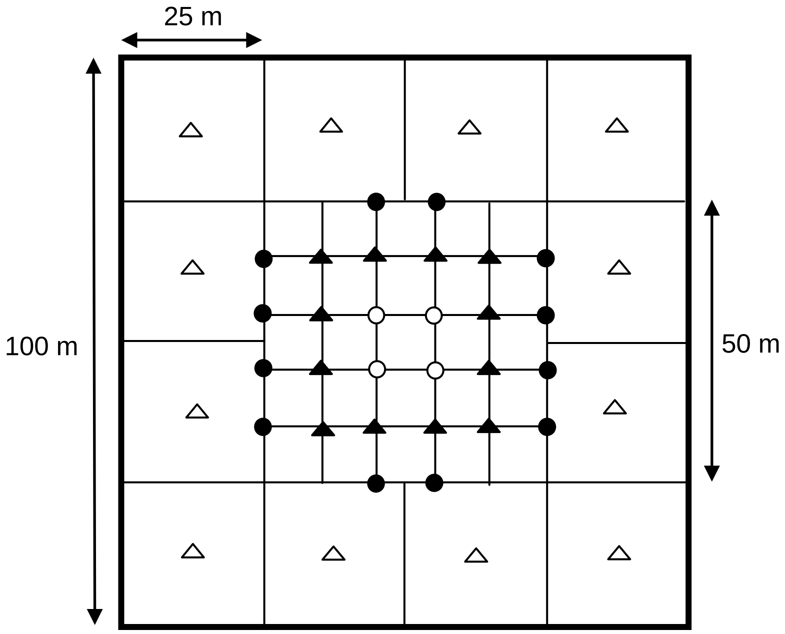

Figure 1. Layout of the field plots. The central 50 m by 50 m portions of the plots were divided into 25 10 m by 10 m quadrats for the nest surveys. Symbols indicate the locations of bait card monitoring stations, as follows: empty triangles = outer stations, filled circles = middle stations, filled triangles = inner stations, empty circles = central stations.

Efficacy against laboratory colonies

On 10/21/08, I collected ants from three separate nesting complexes in the field in the lower ant population, to establish three laboratory colonies. On 11/7/08, nine more colonies were established from field collections, for a total of 12 laboratory colonies. Each colony contained one queen, a few to several dozen males, between 192 and 674 workers, and between 194 and 807 brood (eggs, larvae and pupae). Each colony was placed in a separate nesting box on the day of collection, which consisted of an open plastic container that had the walls coated with fluon to prevent escape (Fig. 2). Each nest box was provided with a nest chamber (a 50 ml centrifuge tube with 20 ml of water in the end, stopped with cotton, to provide moisture and humidity for the nest environment; the whole tube was wrapped in tin foil to keep it relatively dark inside), access to 25% sugar water solution (also in a centrifuge tube, stopped with cotton), and protein food (scrambled egg and chicken meat). Food was refreshed or replaced every few days as needed. Colonies were allowed to acclimate to the lab environment for at least 24 hours, at which point each colony was randomly offered either 0.5g of Advion IG or Maxforce GIB, while continuing to have access to the sugar water and protein food, for a total of six replicate colonies for each bait type. There were no significant differences in either colony size (two-sample t-test, $t = 0.31$, $P = 0.77$), or ratio of brood to workers (two-sample t-test, $t = 0.18$, $P = 0.86$), among colonies assigned to the two bait types. The pesticidal ant bait granules were presented on index cards in the nest box, and were covered with a second tented index card to prevent exposure of the granules to light. All dead workers found outside the nest chamber in the nest box were removed immediately prior to presentation of the pesticidal ant baits. At four and six days post bait introduction, I removed and counted dead workers and queens in each nest box. I terminated the experiment at 10 days post bait introduction, at which point I counted all live and all dead workers and queens.

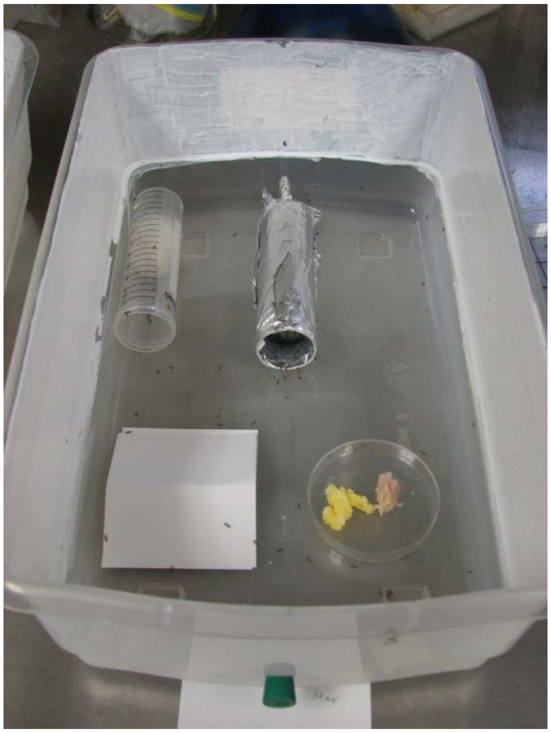

Figure 2. Nesting box housing a laboratory colony. The foil-lined tube is the nesting chamber, while the unlined tube contains 25% sugar water and the petri dish contains chicken meat and scrambled egg. Pesticidal ant bait (either Advion IG or Maxforce GIB) is offered underneath the tented index card.

Bait attractiveness

On 11/13/08 I conducted an open choice bait preference test between Advion IG and Maxforce GIB in the field, at a site with high densities of Argentine ants. I conducted eight replicates, with each replicate containing two index cards placed side by side on the ground; one card contained 1 g of Advion IG while the other contained 1 g of the Maxforce GIB granules (Fig. 3). Each replicate station was separated by at least 5 m from the next. After placing the baits on the cards, I counted numbers of ants on each bait at 5 minute intervals for the first 30 minutes, and then every 10 minutes for the following hour (up to 90 minutes total length).

Figure 3. Open choice bait preference test comparing Advion IG with Maxforce GIB. The image on the left shows a typical arrangement of one of the eight replicates. The image on the right shows a close up view of the replicate bait choice, with Maxforce GIB offered on the left card and Advion IG on the right card.

RESULTS

Field efficacy plots

In both treatment plots, the initial application of Advion IG had no discernable impact on numbers of ants recruited to monitoring bait cards (Fig. 4). In fact, ant numbers increased in both plots (and in the control plot) after the first bait application. This increase in ant numbers in all three plots was most likely due to normal population growth at this time of year (Krushelnycky et al. 2004), although the particularly large increase in the control plot also probably resulted because of an especially low number of ants at monitoring cards on the single pre-treatment monitoring date (Fig. 4). Ant numbers were also very low, for unknown reasons, in all three plots at 34 days after the initial application. The declines in ant numbers in the Advion treated plots at 34 days were almost certainly not due to Advion treatment, for several reasons: 1) numbers in the control plot also decreased on this date, suggesting that some external factor, such as weather conditions, was responsible, 2) indoxacarb is a relatively fast acting toxicant, so any mortality from Advion should have become apparent much sooner, 3) ant numbers rebounded strongly on the next monitoring event (42 days) in the plot that received two Advion applications.

The second application of Advion IG also had no apparent effect on numbers of ants at monitoring bait cards (open circles in Fig. 4). Ant numbers remained high in this plot until the final monitoring event, at 70 days. In contrast, ant numbers at monitoring bait cards declined immediately, and remained low for most of the remainder of the experiment, in the plot that was treated with Maxforce GIB on the second application (Fig.4, closed triangles). Ant numbers appeared to show a slight rebound after the Maxforce application, at 63 days post initial application (Fig. 4), and this likely resulted from foraging or re-colonization from the plot borders. Unlike earlier dates, numbers of ants at 63 days were noticeably higher at outer station monitoring bait cards (located 12.5 m from the plot perimeter), compared to middle or inner station bait cards (Fig. 5). At 70 days post initial application, however, ant numbers had again declined in this plot. Weather may have been partly responsible for the lower numbers in all plots on the final monitoring date. Ant numbers fluctuated moderately in the control plot throughout the experiment, with the exception of the dates mentioned above.

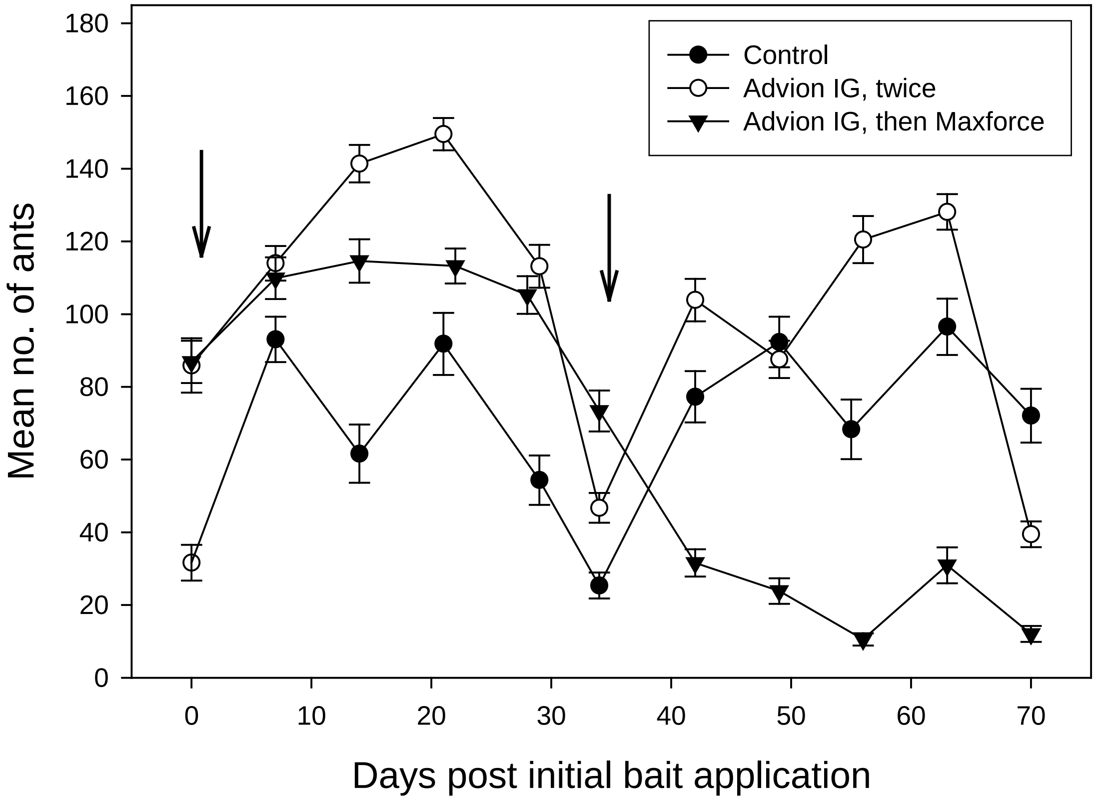

Figure 4. Mean numbers of ants (±1 SE) at monitoring bait cards (n=40) in the three plots during the course of the experiment. Arrows indicate timing of pesticidal ant bait applications.

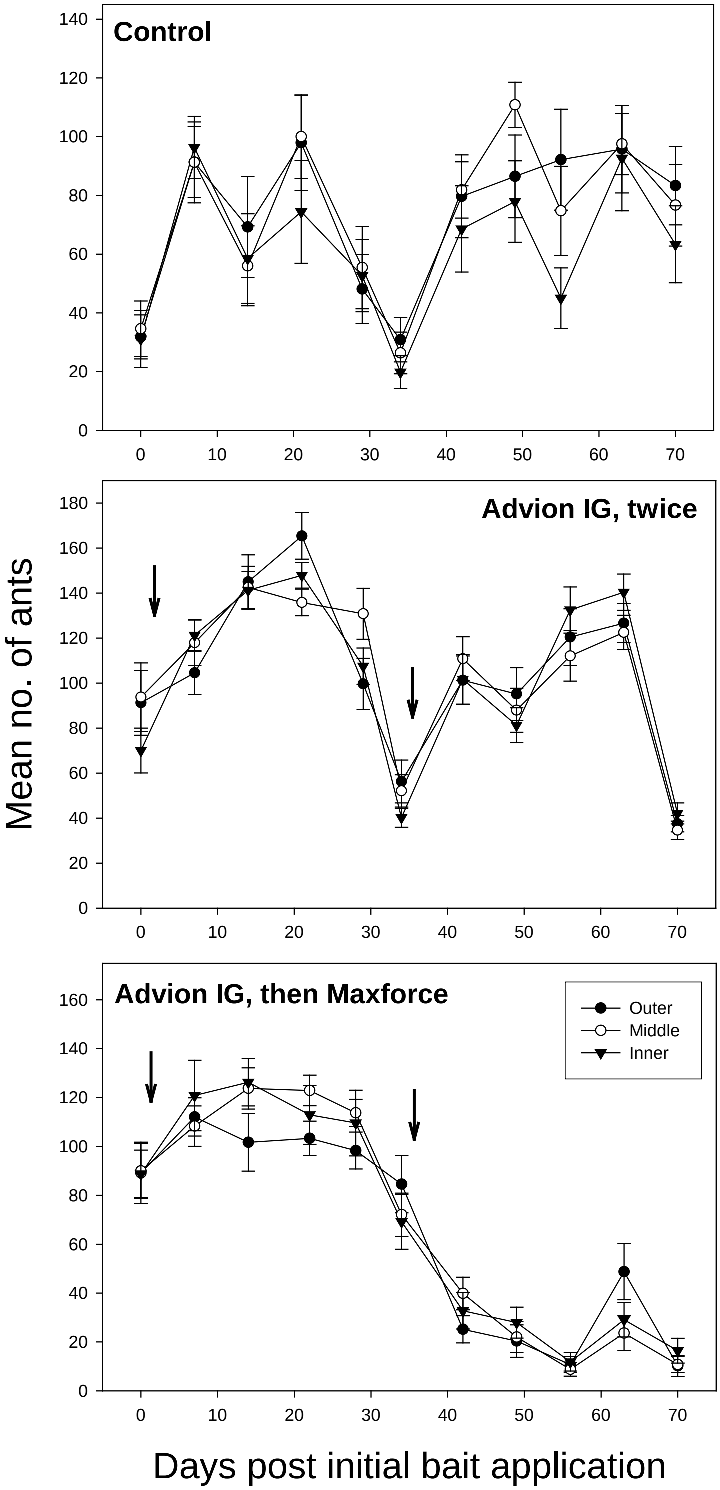

Figure 5. Mean numbers of ants (±1 SE) at three of the four bait card monitoring station types in each of the three plots during the course of the experiment. Arrows indicate timing of pesticidal ant bait applications. See Methods and Figure 1 for the relative positions of the three monitoring station types within the plots.

Nest surveys at two and four weeks after the first application confirmed that a substantial number of nests or nest fragments survived in the central portions of both treatment plots (Figs. 6-9). Nest survey data need to be interpreted with caution due to the high natural spatial variability in nest density. Because a different quadrat was surveyed during each monitoring event, differences between monitoring events in densities of nests or abundances of particular castes or life stages potentially represent natural spatial differences as much as or more than they represent temporal trends in these metrics. Elucidating subtle temporal and/or treatment-induced effects in the nest survey data is therefore difficult. However, nest surveys are highly effective for confirming or discounting a dramatic result, such as eradication, that might be suggested from bait card monitoring. After the first application, queens, eggs, worker larvae, and hence reproductive nests, were all present and fairly abundant in both treated plots, relative to the control plot, clearly indicating that eradication had not occurred. The large decline in number of reproductive nests in one of the Advion-treated plots (Fig. 6) was due to an unusually high number of nests in the quadrat surveyed prior to the first application.

Similarly, nest surveys conducted at one, three and five weeks after the second bait application once again clearly indicated that eradication was not achieved in the experimental plots (Figs. 6-9). Queens, eggs, worker larvae and reproductive nests were all present in both treated plots, regardless of whether the second application was Advion IG or Maxforce GIB.

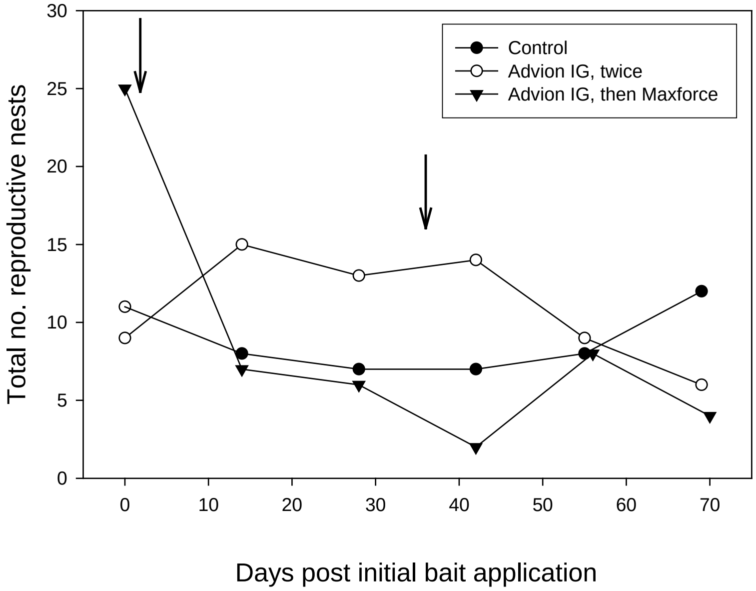

Figure 6. Total number of reproductive nests per monitoring quadrat in the three plots during the course of the experiment. Arrows as in Fig. 4.

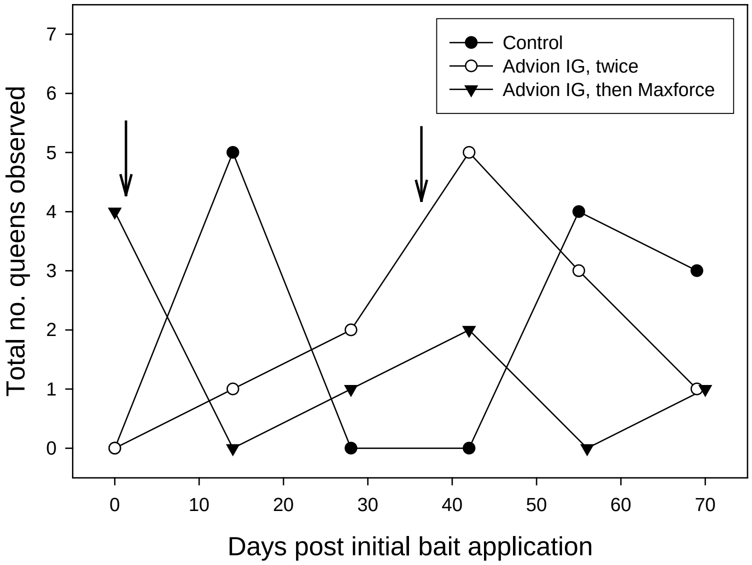

Figure 7. Total number of queens observed per monitoring quadrat in nest surveys in the three plots during the course of the experiment. Arrows as in Fig. 4.

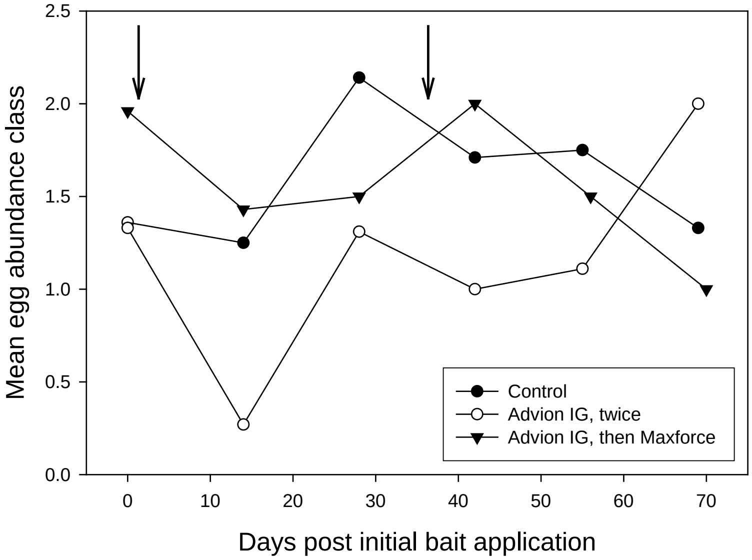

Figure 8. Mean egg abundance class of reproductive nests during nest surveys in the three plots during the course of the experiment. Arrows as in Fig. 4.

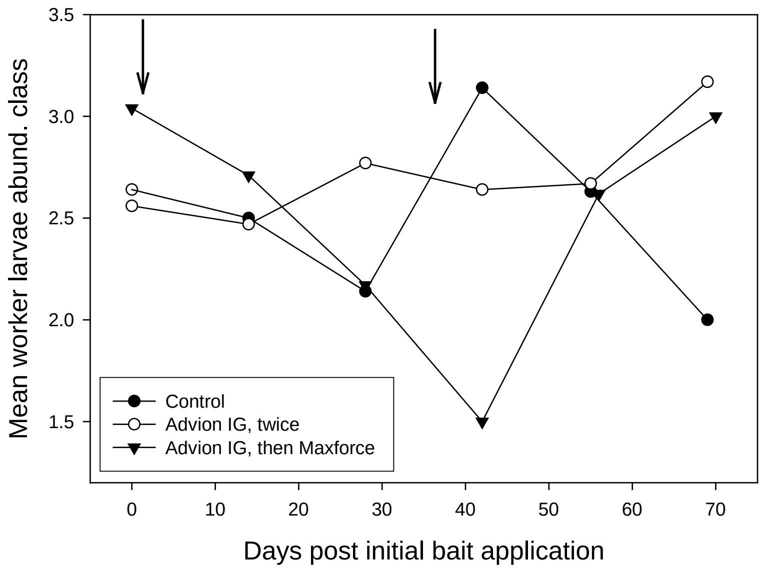

Figure 9. Mean worker larvae abundance class of reproductive nests during nest surveys in the three plots during the course of the experiment. Arrows as in Fig. 4.

Efficacy against laboratory colonies

There were strikingly different patterns of worker ant mortality among laboratory colonies offered Advion IG versus Maxforce GIB (Fig. 10). Worker ant mortality commenced quickly and reached a mean of 94.3% by 10 days after Advion IG was offered to laboratory colonies. In contrast, there was virtually no worker mortality for the first six days after Maxforce GIB was offered to laboratory colonies, and worker mortality only reached an average of 11.4% at the end of the experiment, 10 days after the bait was introduced. Percent mortality of workers after 10 days was significantly different between the two bait types (two-sample t-test, $t = 17.30$, $P < 0.001$).

Queens died in two of the six colonies offered Advion IG, while none of the queens died in the six colonies offered Maxforce GIB. There was no significant difference in the proportion of queens that died between the two treatment types (chi-square contingency table, chi-square = 2.40, df = 1, $P = 0.121$).

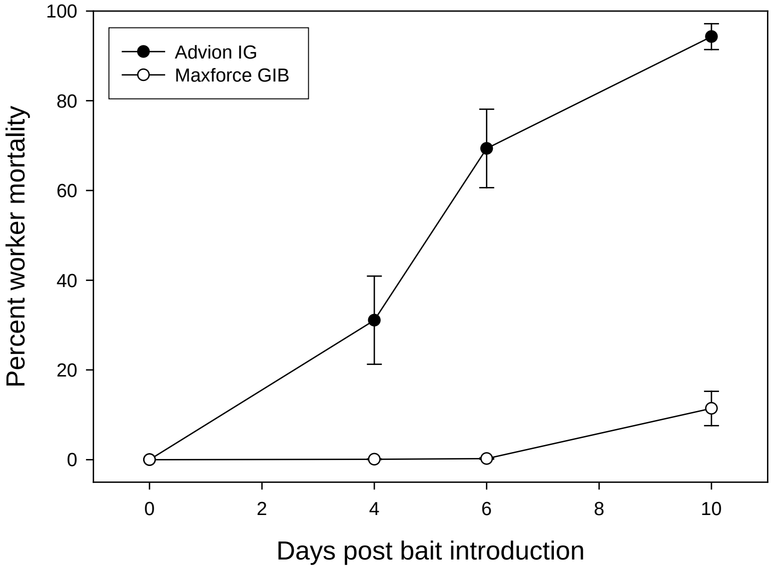

Figure 10. Mean percent mortality of worker ants (±1 SE) over the course of 10 days in laboratory colonies offered either Advion IG or Maxforce GIB (n = 6 colonies per bait type).

Bait attractiveness

Maxforce GIB consistently attracted more ants than did Advion IG during the field bait preference test (Fig. 11). A paired t-test conducted on the mean counts of the eight replicate trials found that the number of ants attracted to the Maxforce granules (5.88 ± 0.67, mean ±1 SE) was significantly higher than the number attracted to Advion granules (1.51 ± 0.25; $t = 6.90$, $P < 0.001$). Numbers of ants were quite low for both types of baits, relative to numbers of ants attracted to the monitoring baitcards in the experimental field plots (Figs. 4 and 5), however this was partly due to the fact that handling time was much lower for the two granular baits. Ants could quickly find and remove bait granules from the bait cards, thus preventing a large buildup of ants on the cards. Both types of granules were observed to be carried off by ants, however it appeared that many more Maxforce granules were removed compared to Advion granules.

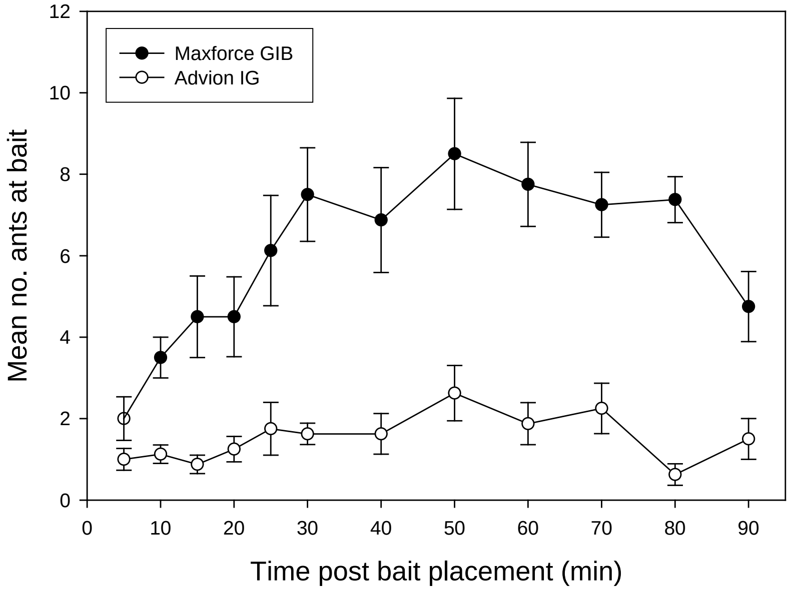

Figure 11. Numbers of ants attracted to Advion IG versus Maxforce GIB over the course of 90 minutes in side-by-side bait preference trials. Data shown are the means (± 1 SE) of eight replicate trials.

DISCUSSION

Based on the results from the experimental field plots, Advion IG does not appear to be an effective bait product for eradicating or even controlling Argentine ants in natural area settings in Hawaii, at least at the application rate tested. Two consecutive applications of Advion at HALE failed to produce a reduction in ant numbers (worker numbers typically increased in the weeks following treatment), and numerous active and reproductive nests persisted at the end of the experiment. When a single application of Advion IG was followed by an application of Maxforce GIB, worker numbers declined in a manner similar to previous experiments using Maxforce (e.g., Krushelnycky and Reimer 1998b, Krushelnycky et al. 2004), yet numerous reproductive nests still persisted in the test plot. The temporary reduction in worker numbers was likely due entirely, or nearly entirely, to the Maxforce application, and not to the combination of both baits.

The Advion IG label allows for broadcast application rates of between 56.1 and 224.5 kg/ha (50 to 200 lbs/acre) on managed turfgrass, or 25 to 100 times the application rate used in this experiment. However, such high application rates would run a considerably greater risk of impacting native nontarget species at HALE due to the much larger amounts of active ingredient available in the environment. Moreover, it seems unlikely that higher application rates would achieve much more control. A related product using the same active ingredient, Advion Fire Ant Bait, is labeled for use at 1.68 kg/ha, a rate lower than the one used here, and most other granular ant baits are labeled for use at broadcast rates of 1.68 to 2.24 kg/ha (1.5 to 2 lbs/acre). If Advion

IG was highly attractive to Argentine ants at HALE, it should have been much more effective at the application rate tested.

Insufficient attractiveness of the Advion IG granules to HALE Argentine ants was suggested by the field bait preference test, in which Advion IG granules were much less attractive than Maxforce GIB granules. These results matched the results in the 1 ha field plots, where a single Maxforce GIB application yielded substantial control of ant workers while none of the Advion IG applications resulted in worker number declines. However, both of these results conflicted strongly with the laboratory colony experiment, which produced opposite results. Colonies offered Advion IG suffered over 90% worker mortality after 10 days, whereas those offered Maxforce GIB lost less than 15% of workers. These outcomes were remarkably consistent between all replicate colonies within the same treatments. The high mortality in the Advion IG colonies was similar to that reported for laboratory colonies of red imported fire ants that were offered Advion Fire Ant Bait (Oi and Oi 2006). However, the Argentine ants in the present study were not seen carrying Advion IG granules back to the nest chambers, and no granules were found in the nest chambers, suggesting that the ants hadn’t ingested the bait. Instead, ants may have received a toxic dose through topical exposure while investigating and contacting the granules and subsequent self-cleaning. The level of incidental exposure of worker ants to granules broadcast in the field is undoubtedly dramatically lower, and may explain the lack of observed mortality in the field plots. If mortality among laboratory colony workers resulted primarily from contact exposure, this could also explain the lower level of mortality among queens in the Advion colonies.

The nearly complete lack of mortality in colonies offered Maxforce GIB is more difficult to explain. This bait has consistently killed large numbers of worker ants when broadcast in the field, as it did in the field plots in this study, typically in less than one week. In this case the laboratory colonies had access to competing food sources, both protein and sugar, which appeared to be preferred over the Maxforce granules: as in the Advion colonies, ants were not seen carrying Maxforce granules back to the nest chambers. Mortality may have increased with colony starvation and over a longer time period (Oi and Oi 2006). Colony starvation and a lack of competing food resources, however, would not seem to approximate normal conditions of ants in the field. It may also be that the unnatural conditions of the laboratory colonies led to altered foraging behavior among the ants.

Conflicting results between trials conducted at different scales and testing different aspects of a bait’s performance has become a recurring theme in research on ant bait efficacy against Argentine ants at HALE. For example, several formulations of Gourmet Liquid Ant Bait were found to be quite attractive in bait preference tests but were then largely ignored when placed in bait stations in experimental plots (Krushelnycky 2008a). Similarly, a corn grit/soybean oil-based granular bait (Advance Granular Carpenter Ant Bait) that was extremely attractive in a bait preference test yielded little control when applied in experimental plots (W. Haines unpubl. data). In a third example involving 0.5 HP Ant Bait, a blend of two different granular bait carriers, ants preferred one granule type over the other in bait preference tests, but the less attractive granule type appeared to yield slightly greater levels of control when broadcast in field plots (Krushelnycky 2008b). In the present study, results between the field-based bait preference test and large-scale field plots were largely consistent, but conflicted strongly with the laboratory colony trial. Further testing of new products, as they are developed, will be necessary to find a more effective bait for controlling Argentine ants in Hawaii. The inconsistency among results of different types of tests, listed above, suggests that while smaller scale tests (laboratory efficacy, bait-preference tests) provide some information, their results may not necessarily accurately predict outcomes at the largest scale – experimental field plots. Ultimately, relatively large field plots must be used to reliably assess a bait's likely performance for landscape-level control or eradication.

ACKNOWLEDGMENTS

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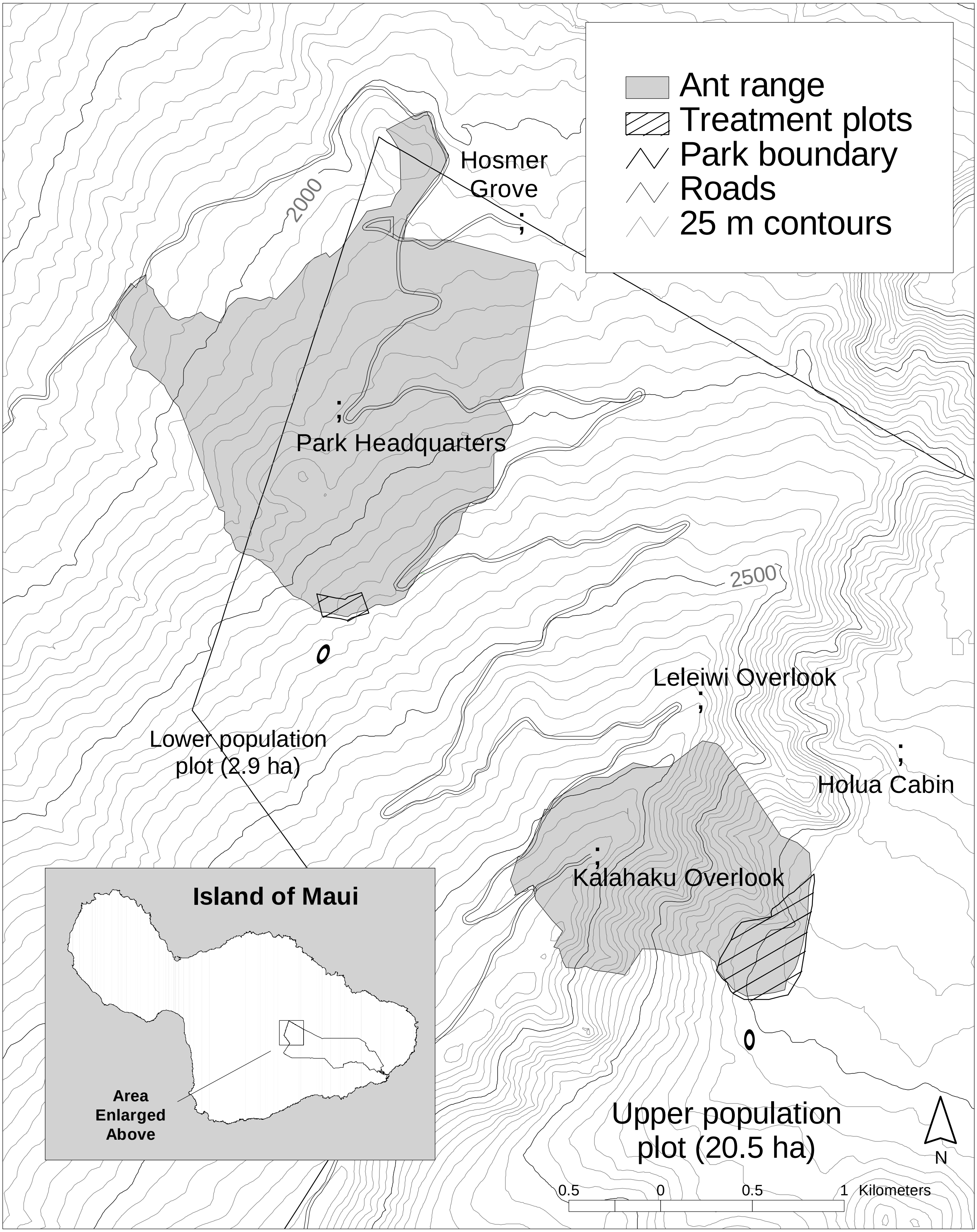
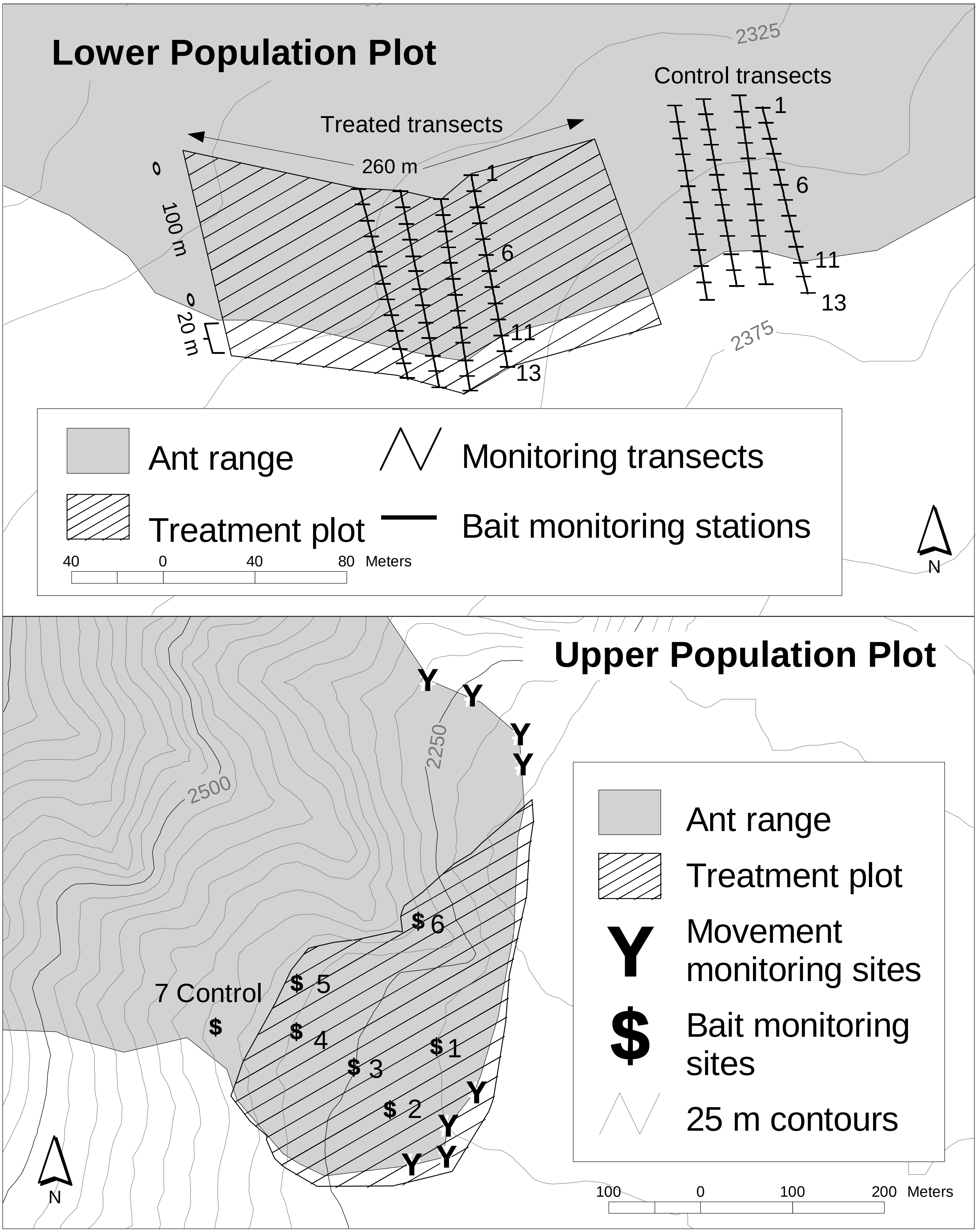
APPENDIX 9. Results of 1996 experimental perimeter plots.

Figures 1 and 2, on the following two pages, show the locations and details of the two 1996 perimeter plots.

APPENDIX 9. Figure 1



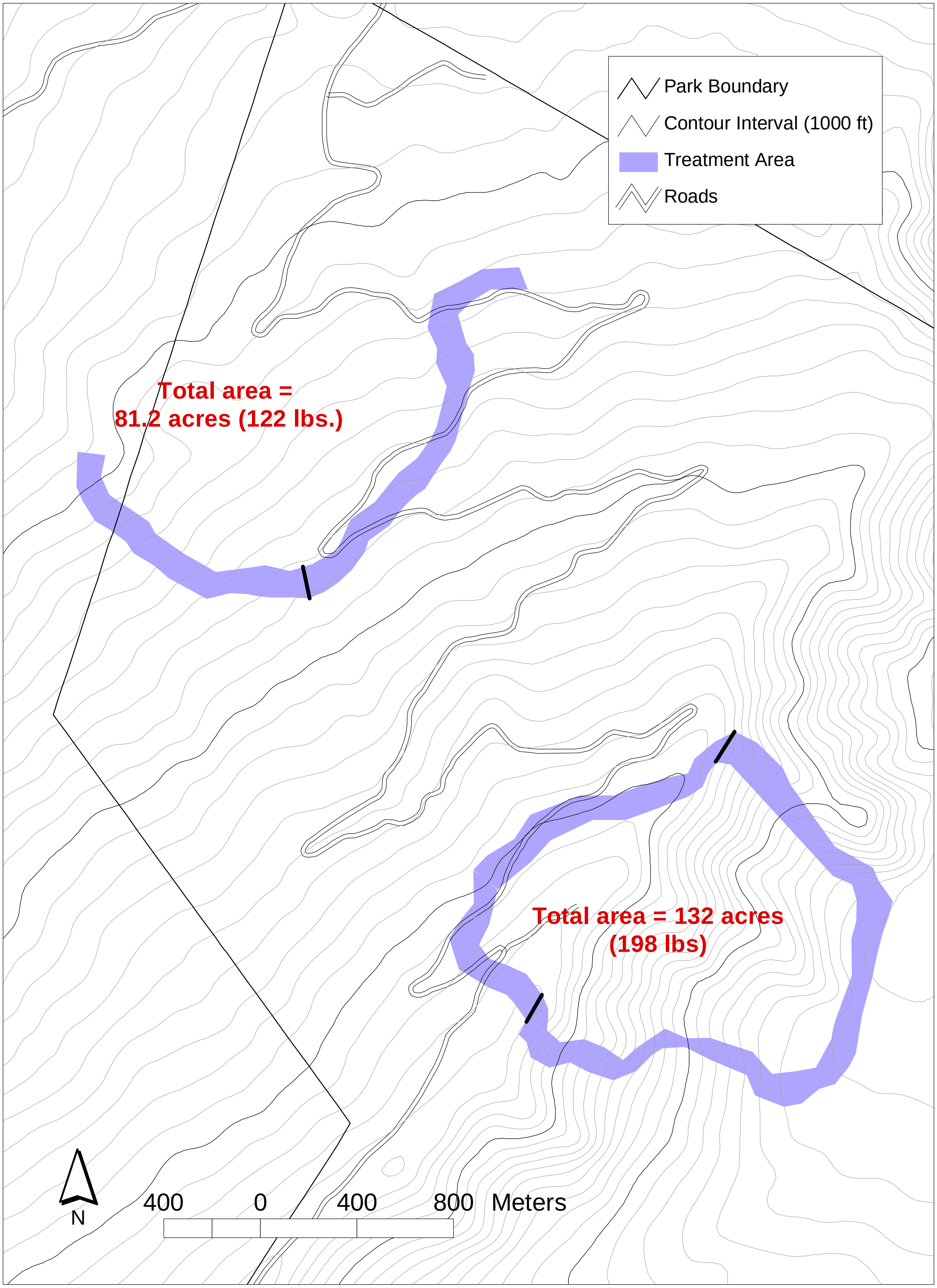
APPENDIX 9. Figure 2



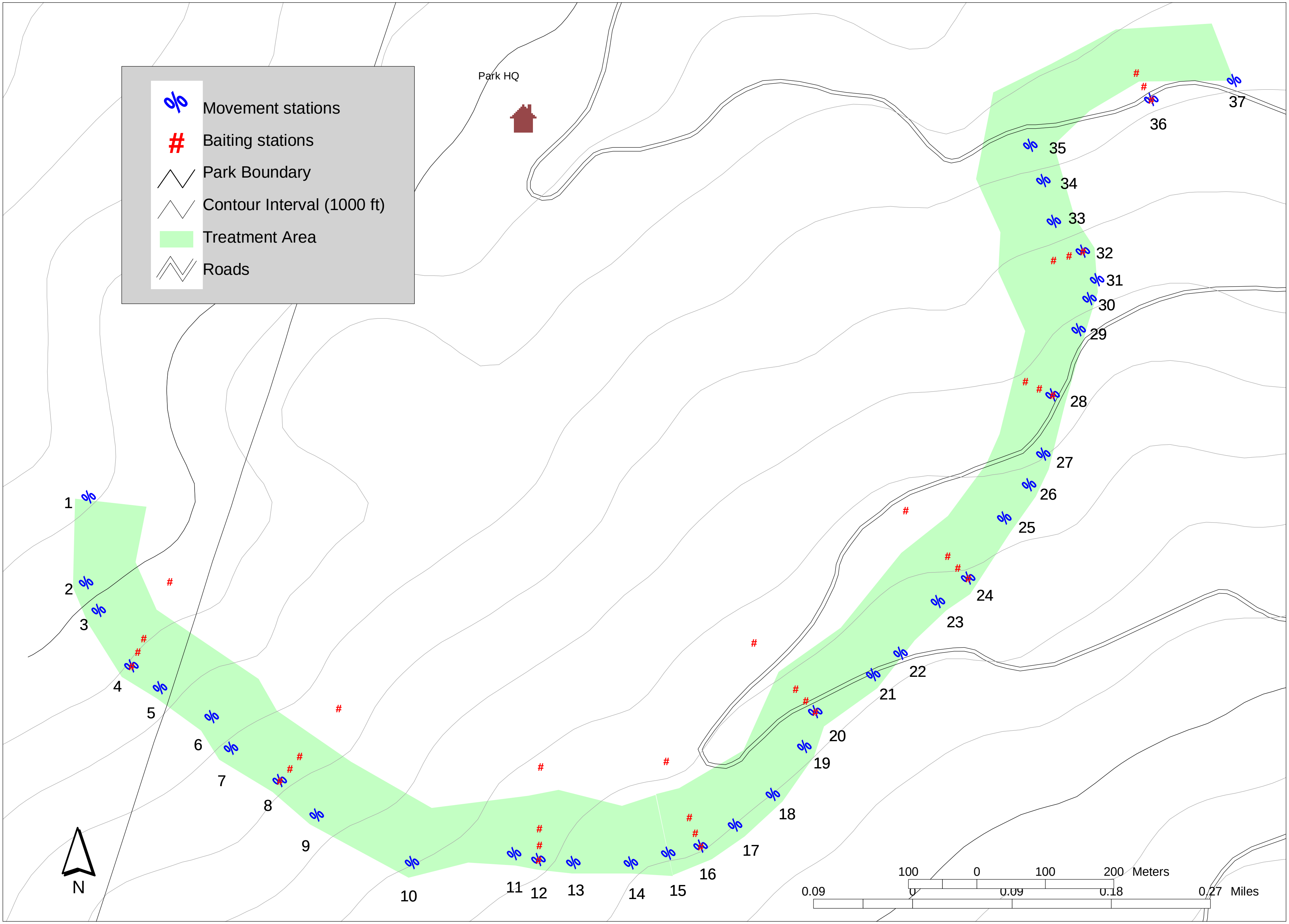
APPENDIX 10. 1997 – 2004 experimental containment treatments.

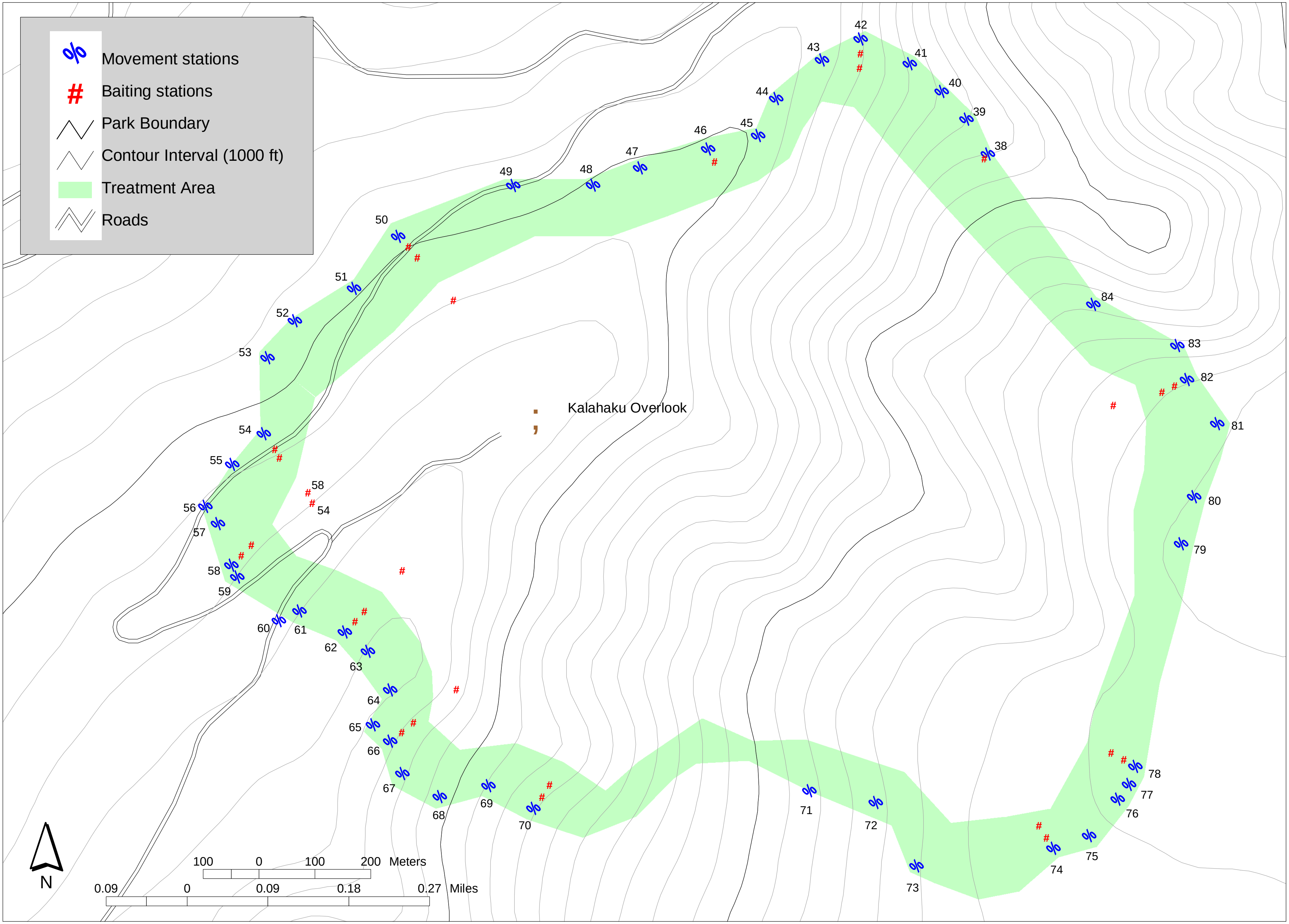
Figures 1 through 3, on the following three pages, show the 1997 perimeter treatment areas and the monitoring station setup for the 1997 perimeter treatment. Perimeter treatment areas and monitoring methods were similar in 1998-2004, but changed slightly to reflect new ant distributions each year.

Appendix 10. Figure 1. 1997 experimental perimeter treatment areas.



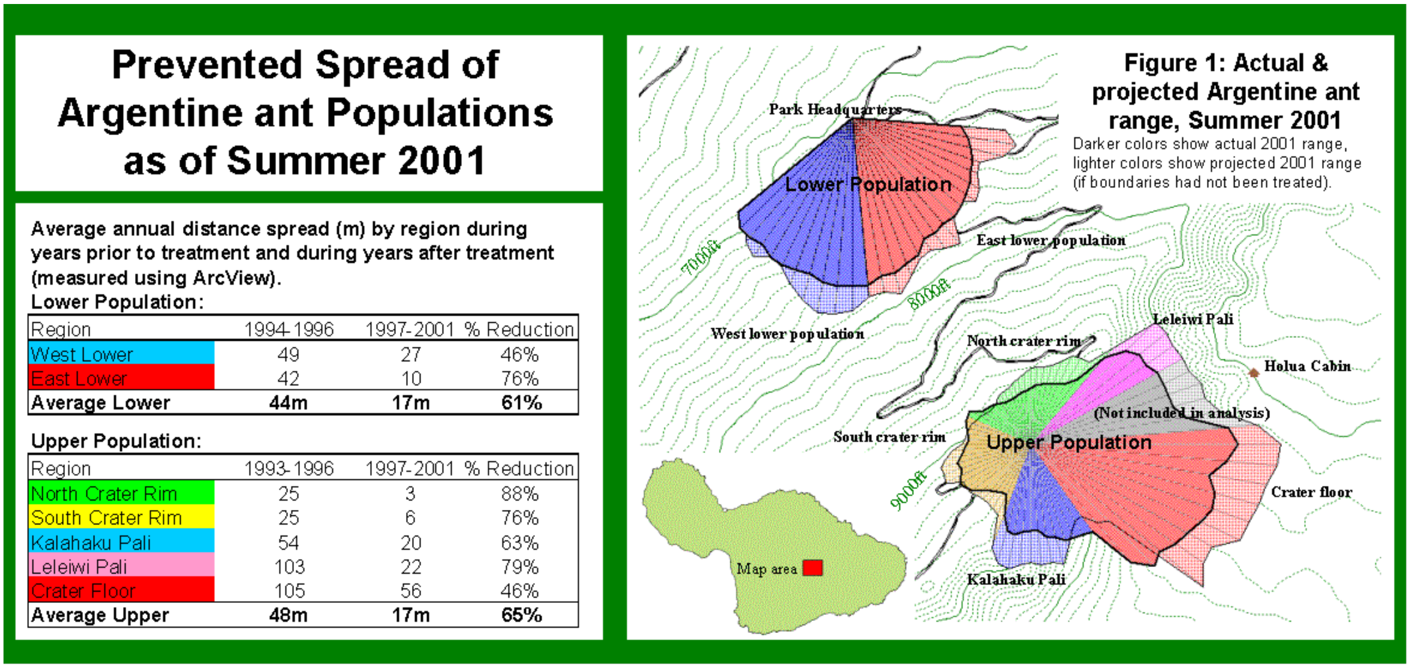
Appendix 10. Figure 2. Monitoring layout for 1997 perimeter area, lower population.





Appendix 10. Figure 3. Monitoring layout for 1997 border treatment area, upper population.

APPENDIX 10. Figure 4. Analysis of perimeter containment effectiveness from 1997 to 2001.



Prevented Spread of Argentine ant Populations as of Summer 2001

Average annual distance spread (m) by region during years prior to treatment and during years after treatment (measured using ArcView).

Lower Population:

Region	1994-1996	1997-2001	% Reduction
West Lower	49	27	46%
East Lower	42	10	76%
Average Lower	**44m**	**17m**	**61%**

Upper Population:

Region	1993-1996	1997-2001	% Reduction
North Crater Rim	25	3	88%
South Crater Rim	25	6	76%
Kalahaku Pali	54	20	63%
Leleiwi Pali	103	22	79%
Crater Floor	105	56	46%
Average Upper	**48m**	**17m**	**65%**

Figure 1: Actual & projected Argentine ant range, Summer 2001

Darker colors show actual 2001 range, lighter colors show projected 2001 range (if boundaries had not been treated).