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

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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 inapropriate 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 commericially 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.

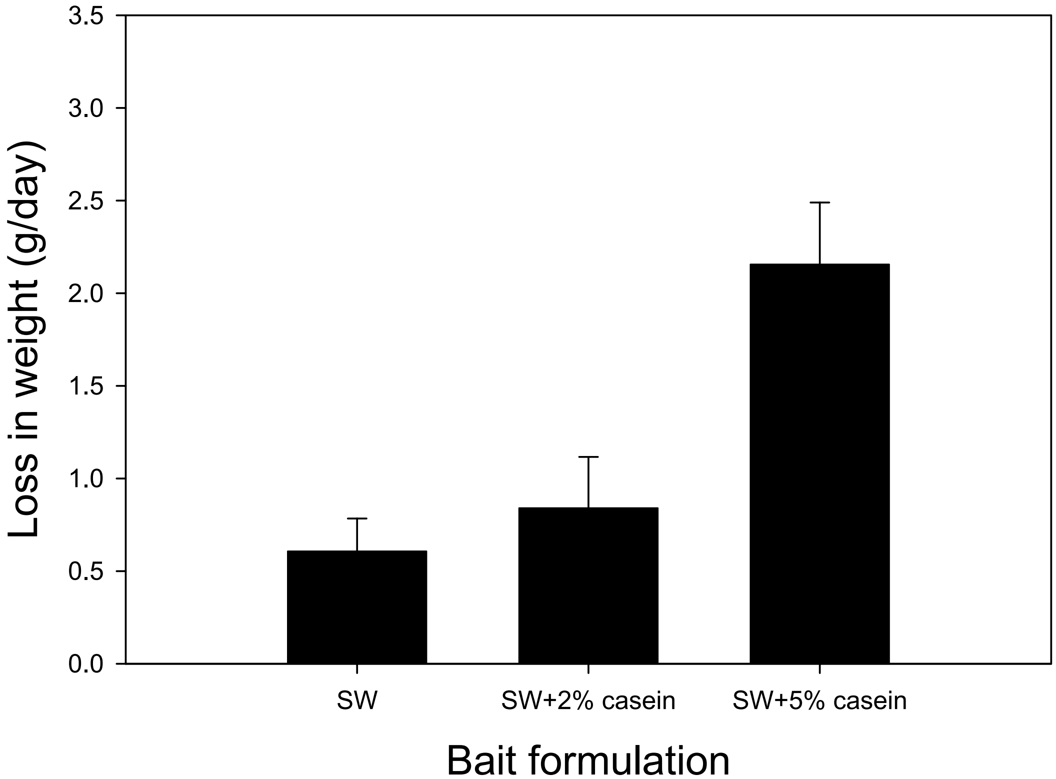

Figure 2. Mean loss in weight per day, over the course of four days, for three bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. SW is 25% sugar water, while formulations with casein used hydrolyzed casein (percent composition is by weight).

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).

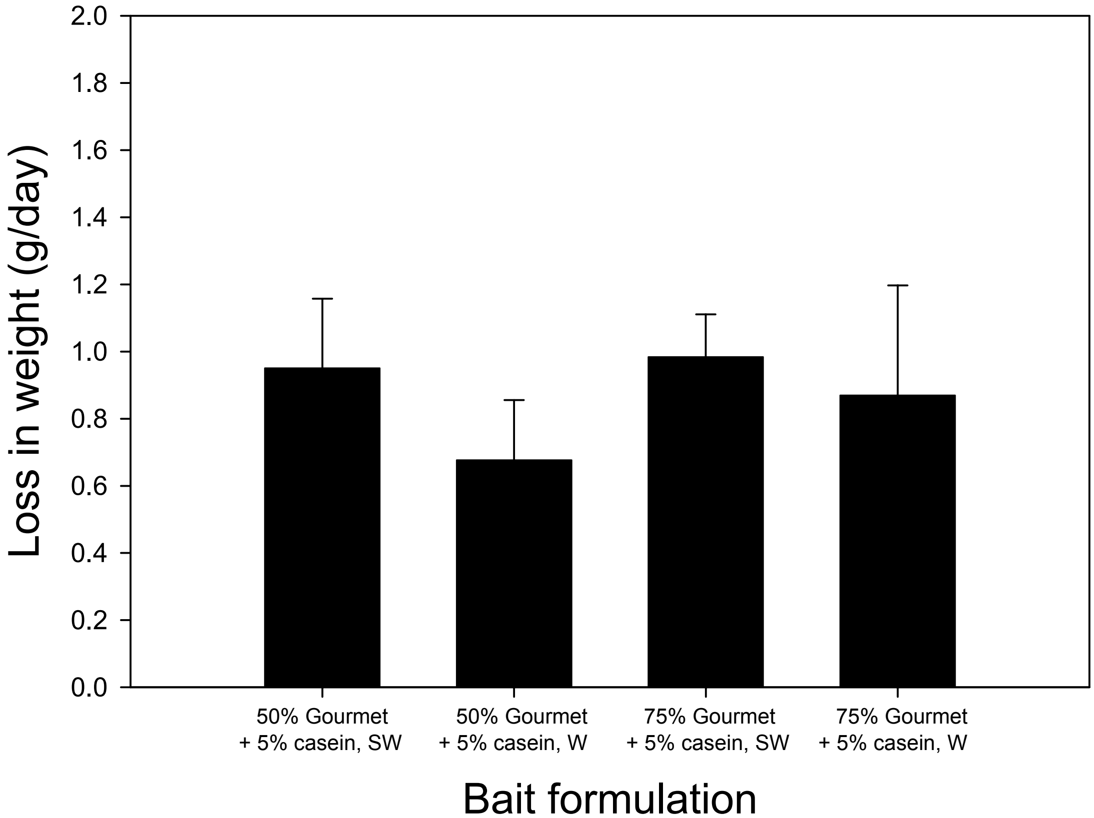

Figure 3. Mean loss in weight per day, over the course of four days, for four Gourmet Liquid Ant Bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. SW indicates formulations were diluted with 25% sugar water, while W indicates formulations were diluted with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

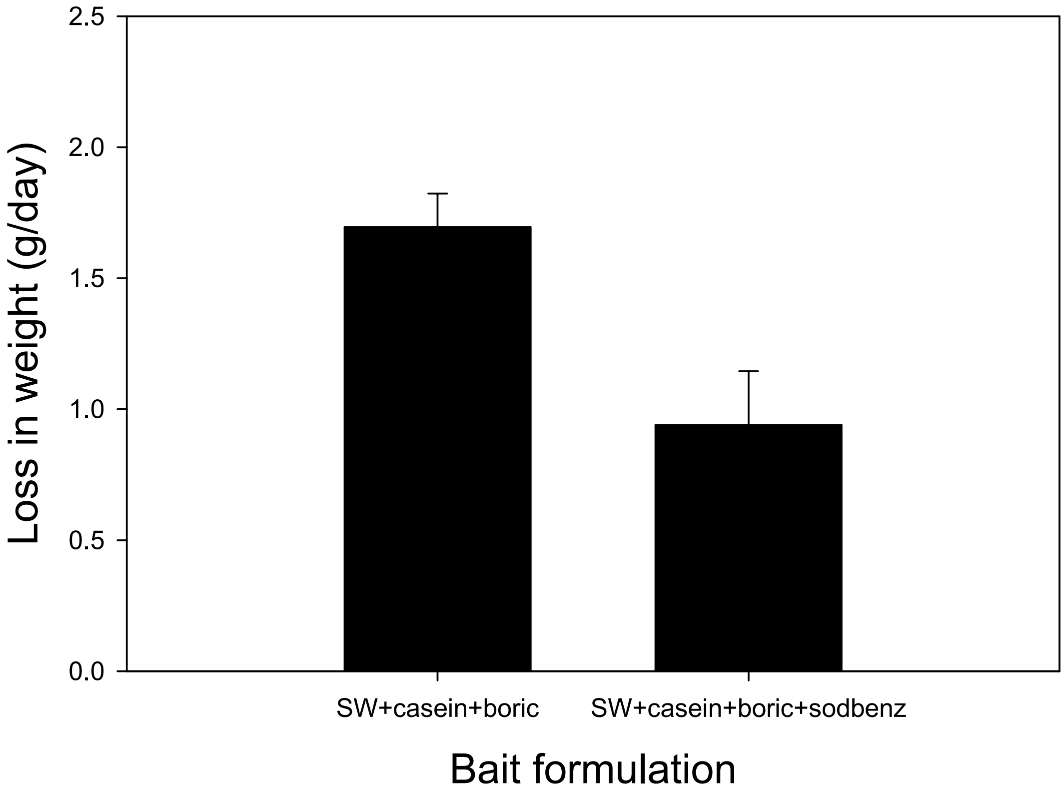

Figure 4. Mean loss in weight per day, over the course of three days, for two sugar water based bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. SW indicates 25% sugar water, casein indicates the addition of 5% hydrolyzed casein (by weight), boric indicates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

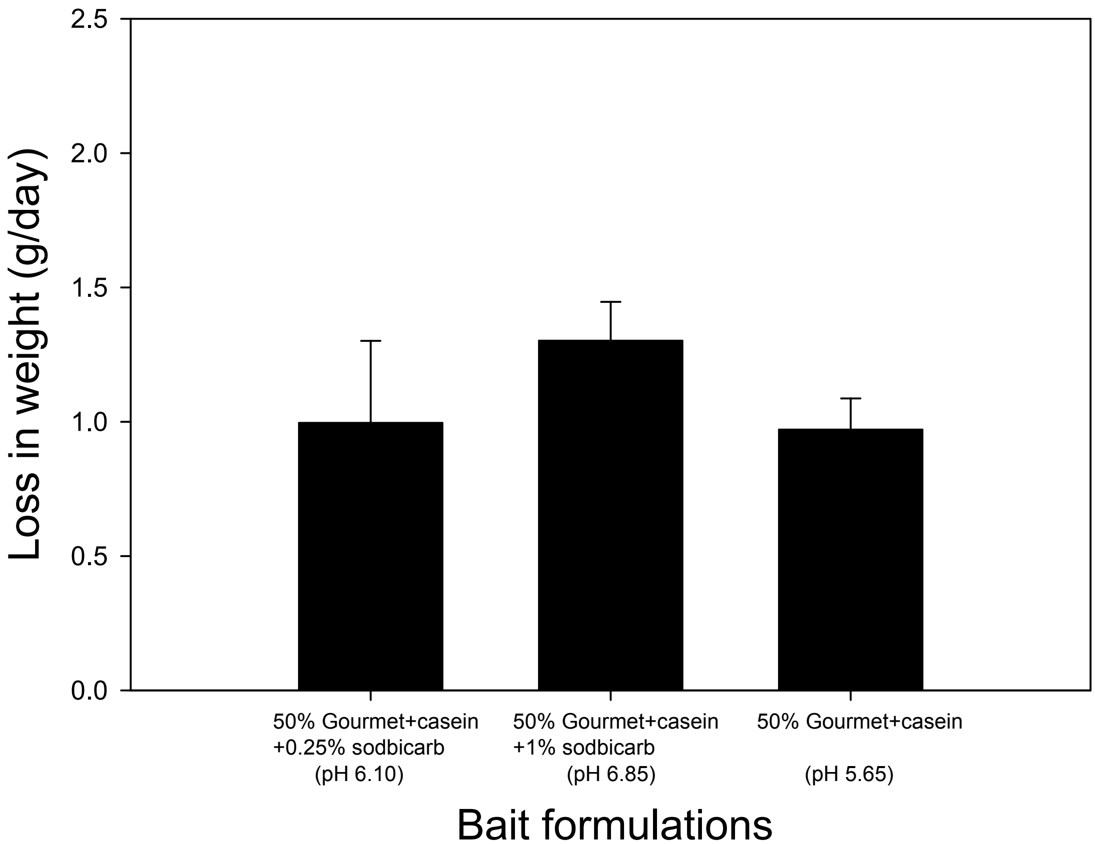

Figure 5. Mean loss in weight per day, over the course of four days, for three Gourmet bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. Casein indicates the addition of 5% hydrolyzed casein (by weight), and sodbicarb indicates the addition of sodium bicarbonate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

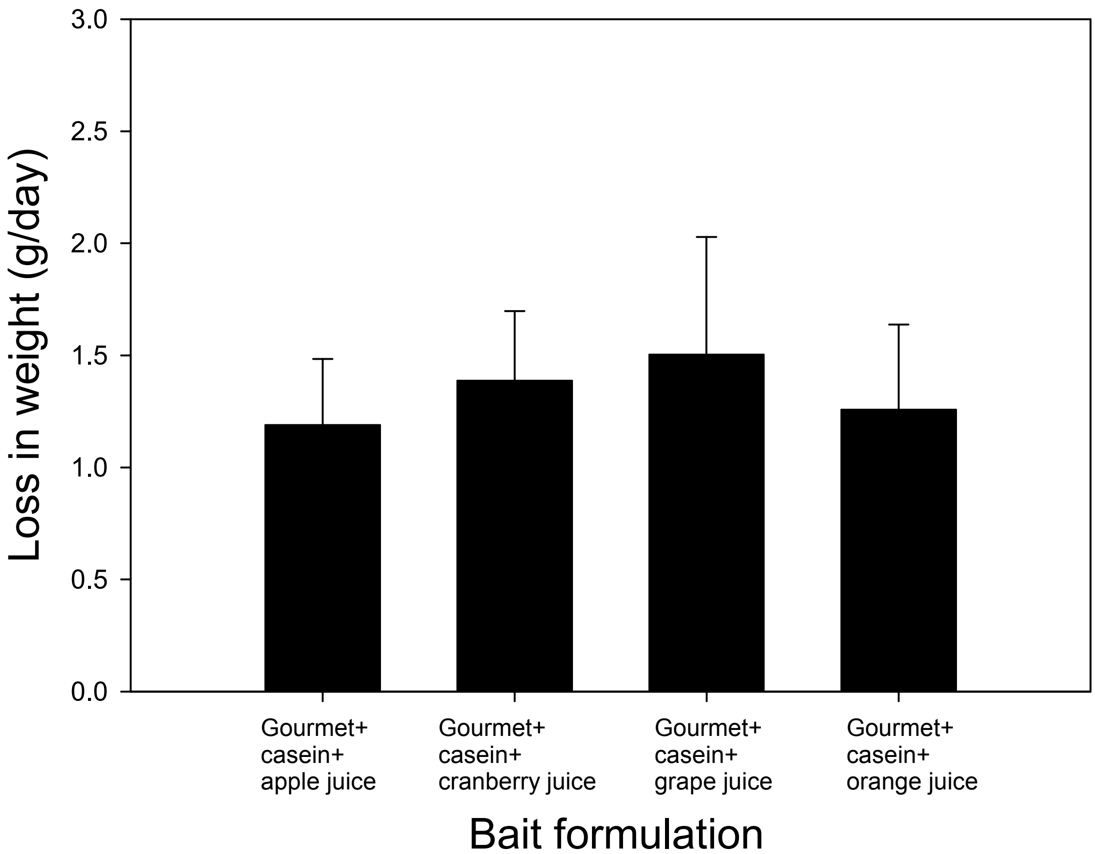

Figure 6. Mean loss in weight per day, over the course of four days, for four Gourmet and fruit juice bait formulations presented to Argentine ants in a choice test. Bars indicate one standard error. All formulations were 50% Gourmet + 5% casein + 45% fruit juice (all percentages by weight).

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.

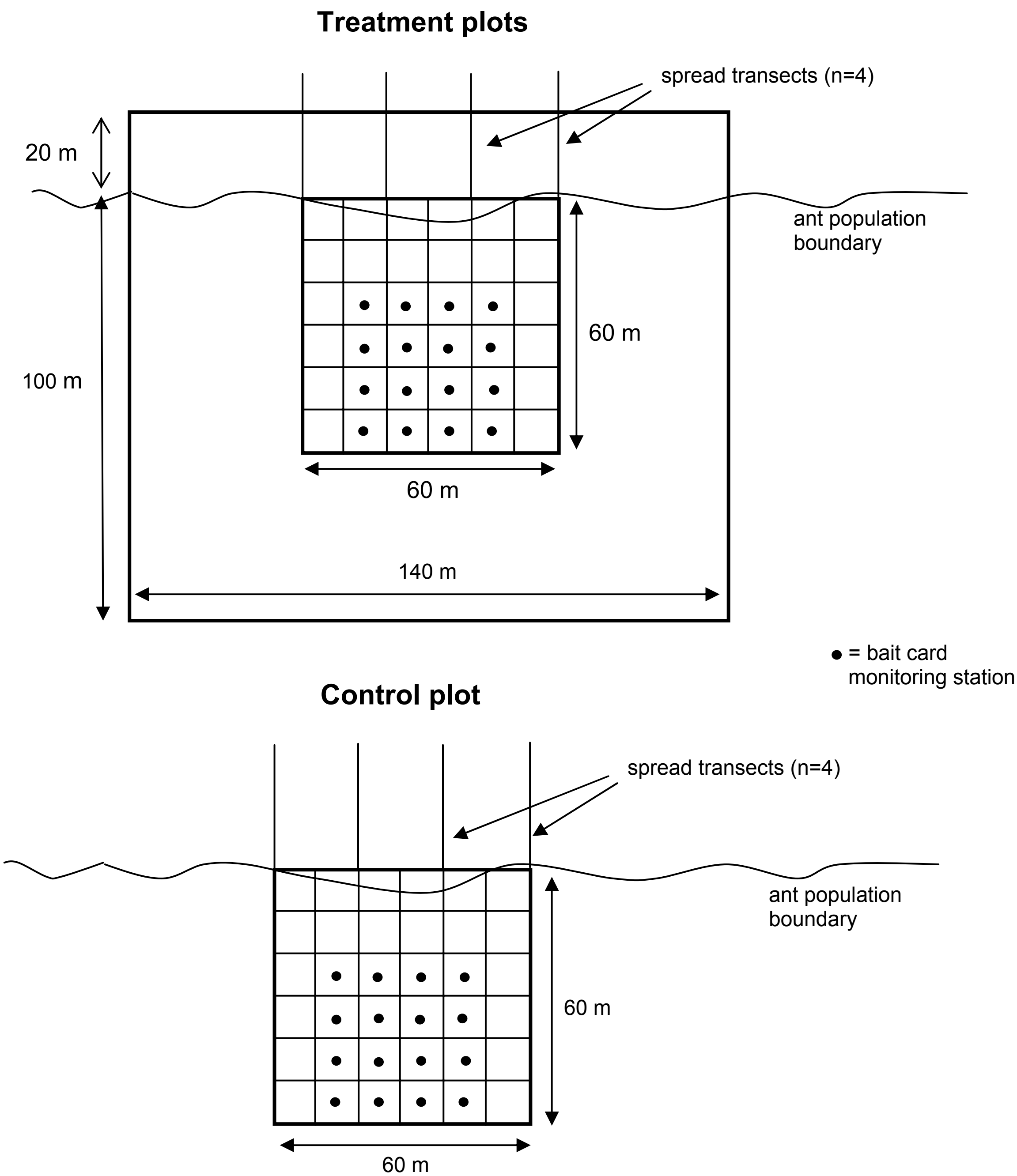

Figure 7. Plot layouts for the two treatment and one control plots. The central monitoring cores in each plot were divided into 36 10 m by 10 m quadrats for nest monitoring. Sixteen bait card monitoring stations in each plot are indicated with dots. Four spread monitoring transects emanated from each 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.

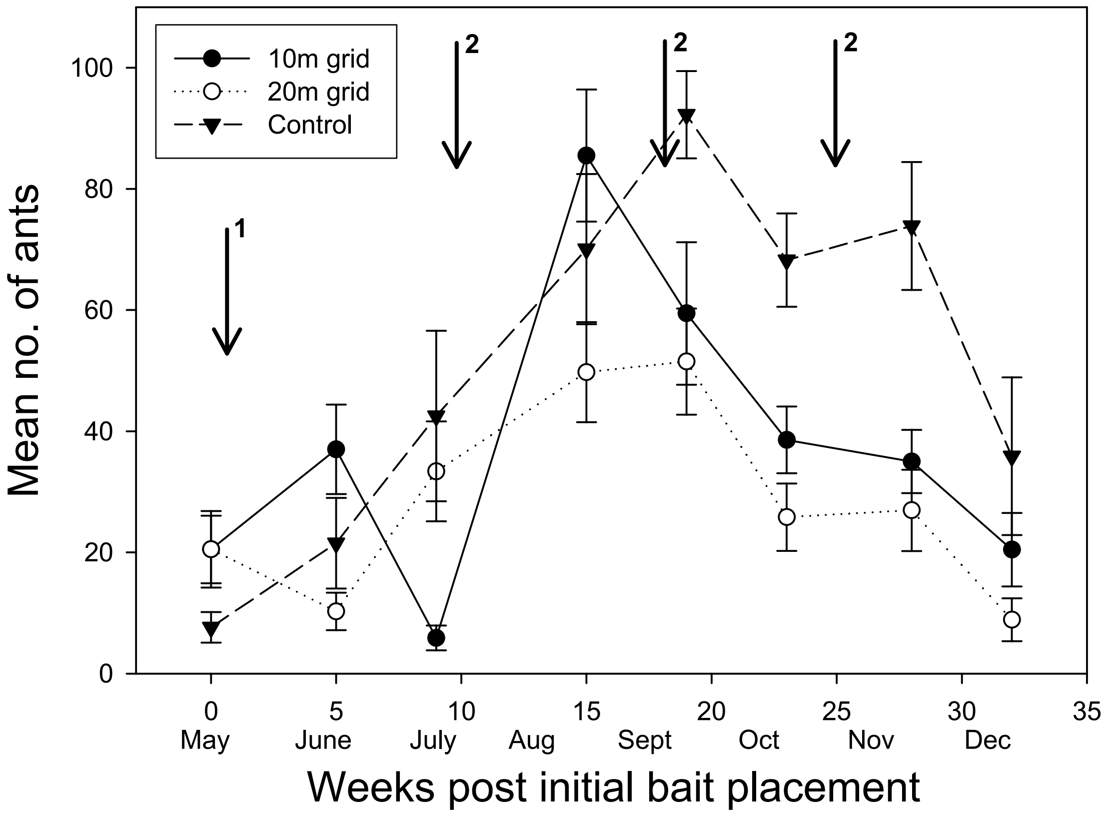

Figure 8. Mean number of ants at bait card monitoring stations in each plot. Bars indicate one standard error. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time (1 = 75% Gourmet + 5% hydrolyzed casein (by weight), diluted 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.

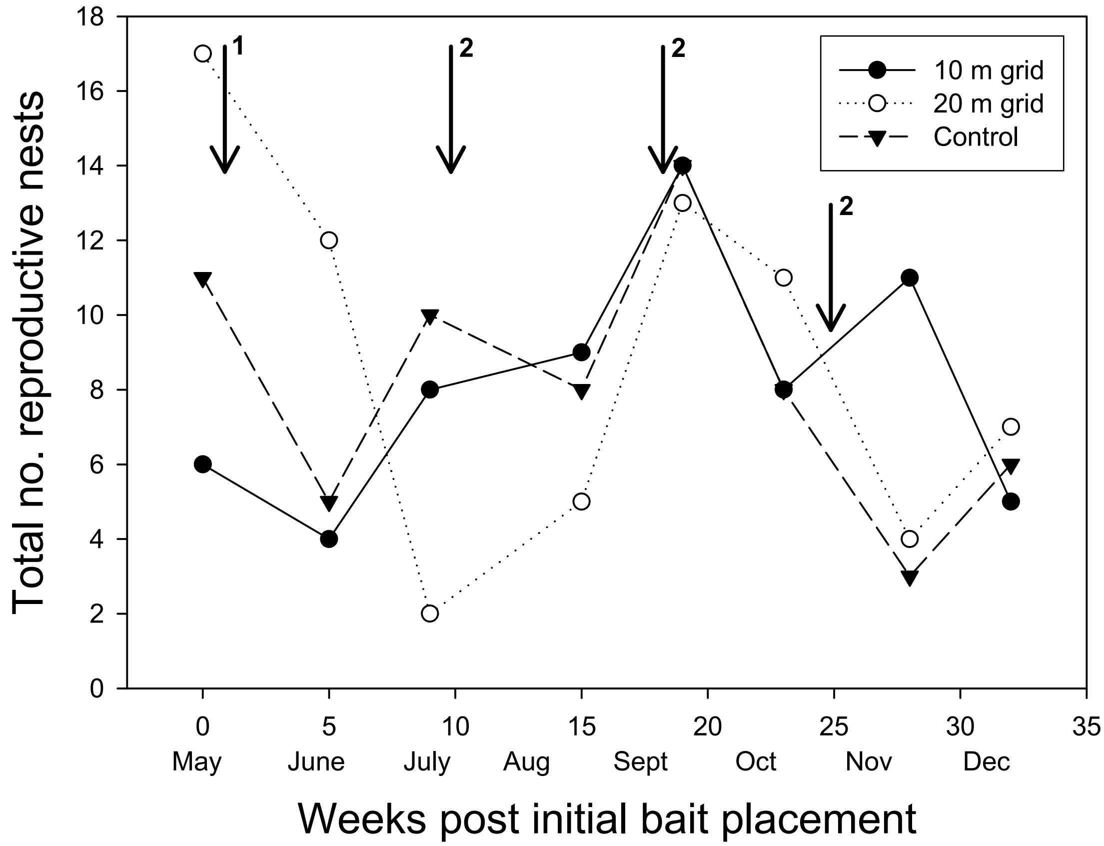

Figure 9. Total number of reproductive nests per monitoring quadrat in each plot. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time (same as in Figure 8).

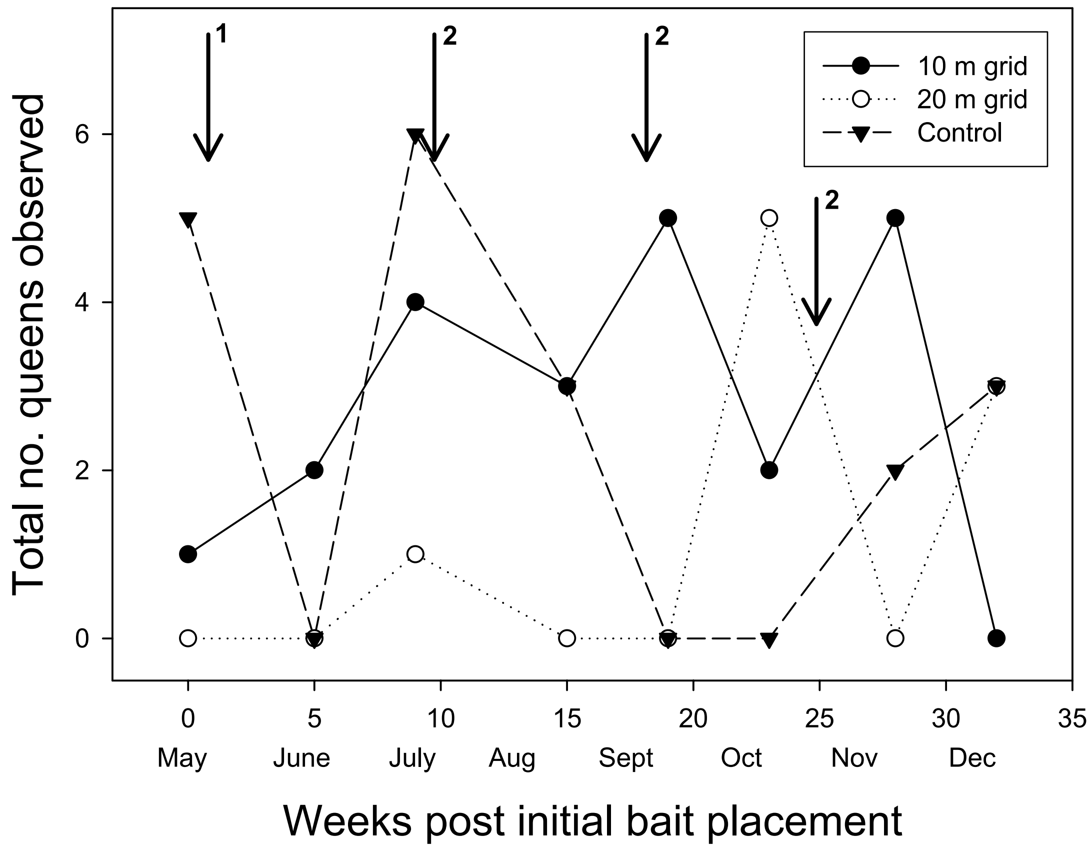

Figure 10. Total number of queens observed per monitoring quadrat during nest surveys in each plot. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time (same as in Figure 8).

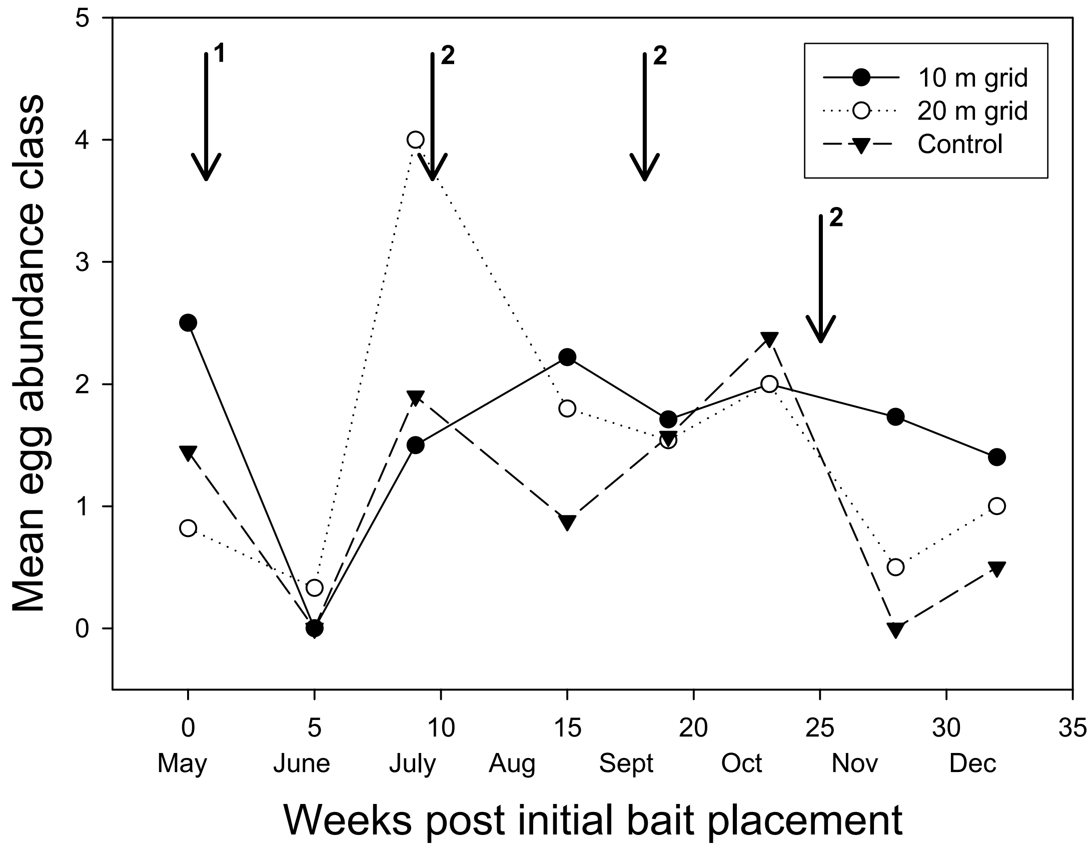

Figure 11. Mean egg abundance class of reproductive nests in each plot. Abundance classes are 0 = 0, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 101-500, 5 = >500. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time (same as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

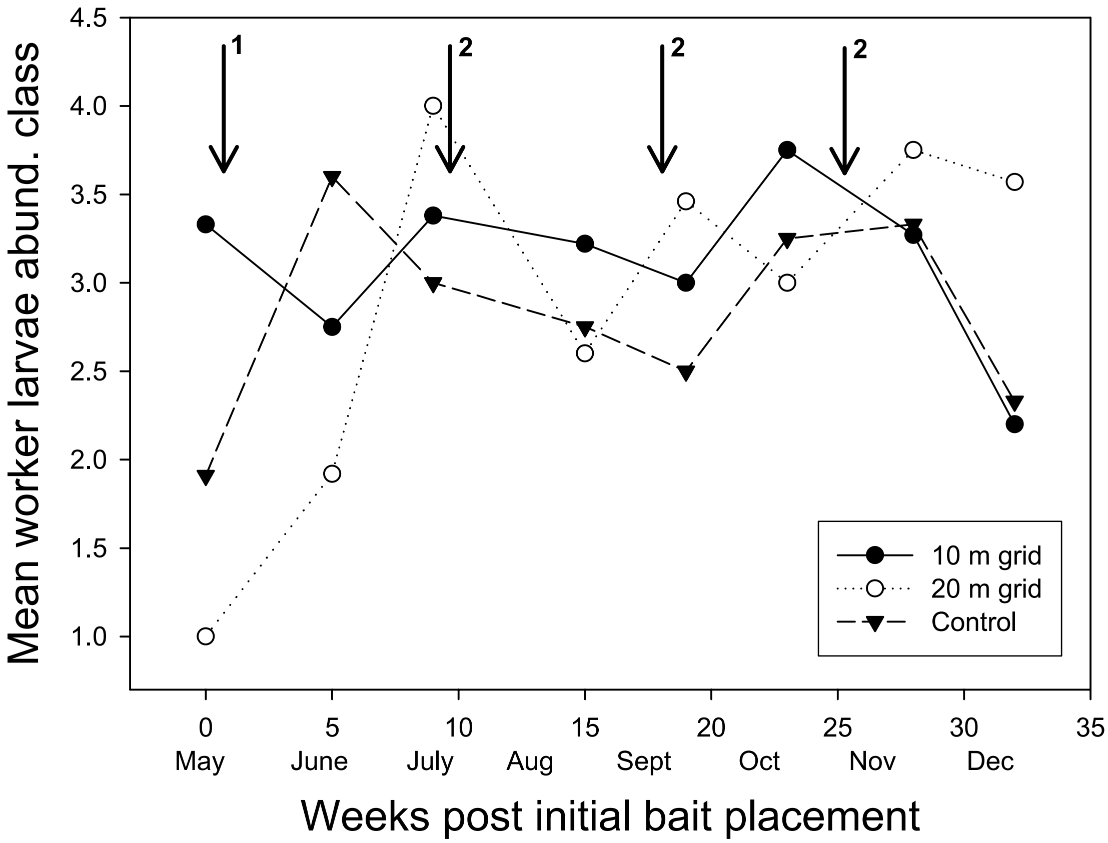

Figure 12. Mean worker larvae abundance class of reproductive nests in each plot. Abundance classes are 0 = 0, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 101-500, 5 = >500. The arrows indicate each time baits were deployed or replaced, and numbers next to arrows indicate which formulation was used each time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

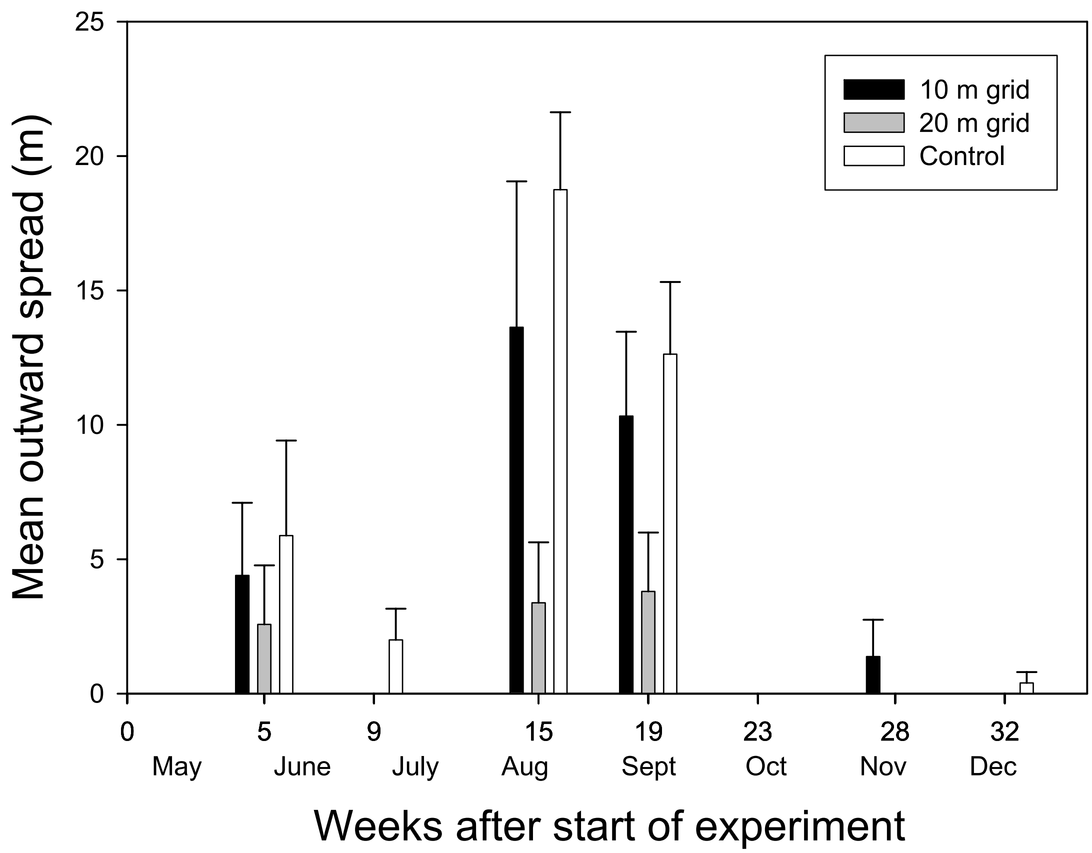

Figure 13. Mean outward spread of the lower ant population at each plot during the interval beginning from the previous monitoring event. For example, spread at week 5 occurred during weeks 0 to 5, and spread at week 9 occurred during weeks 5 to 9. There was no outward spread in any plots during weeks 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).

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