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

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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.

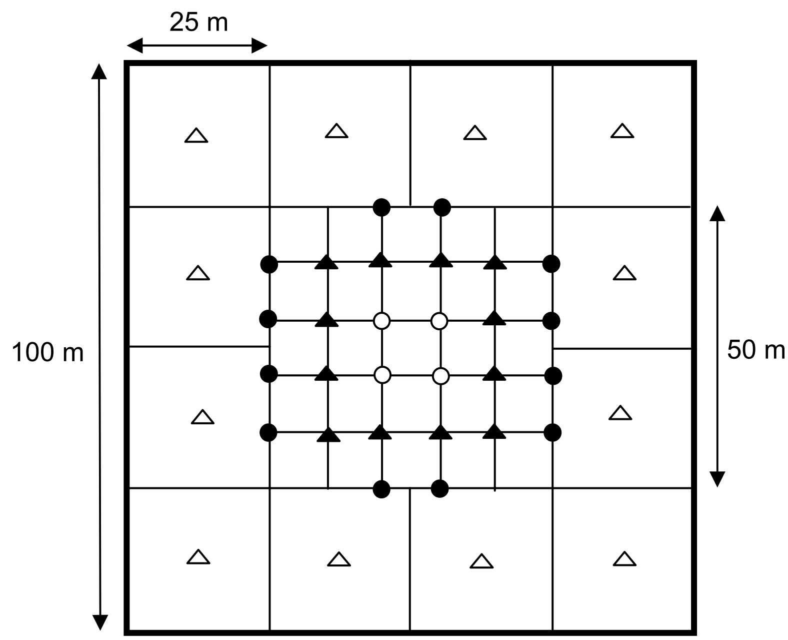

Figure 1. Layout of the plots. The central 50 m by 50 m portions of the plots were divided into 25 10 m by 10 m quadrats for the nest surveys. Symbols indicate the locations of bait card monitoring stations, as follows: empty triangles = outer stations, filled circles = middle stations, filled triangles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

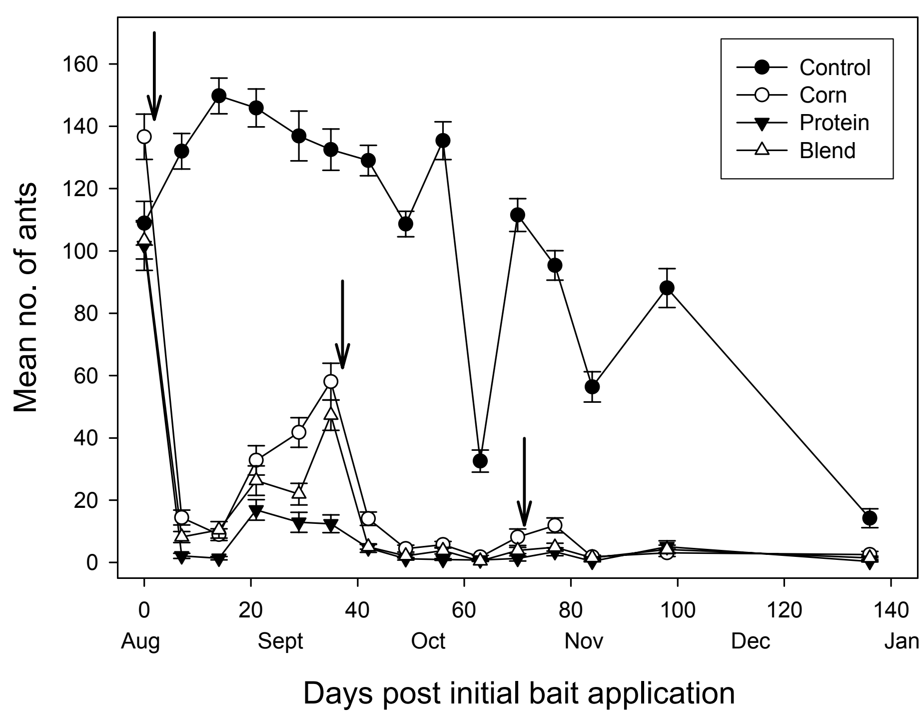

Figure 2. Mean numbers of ants (±1 SE) at monitoring bait cards (n=40) in the four plots during the course of the experiment. Arrows indicate timing of pesticidal ant bait applications. The first two applications were made in all three treatment plots, while the third application was only made in the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

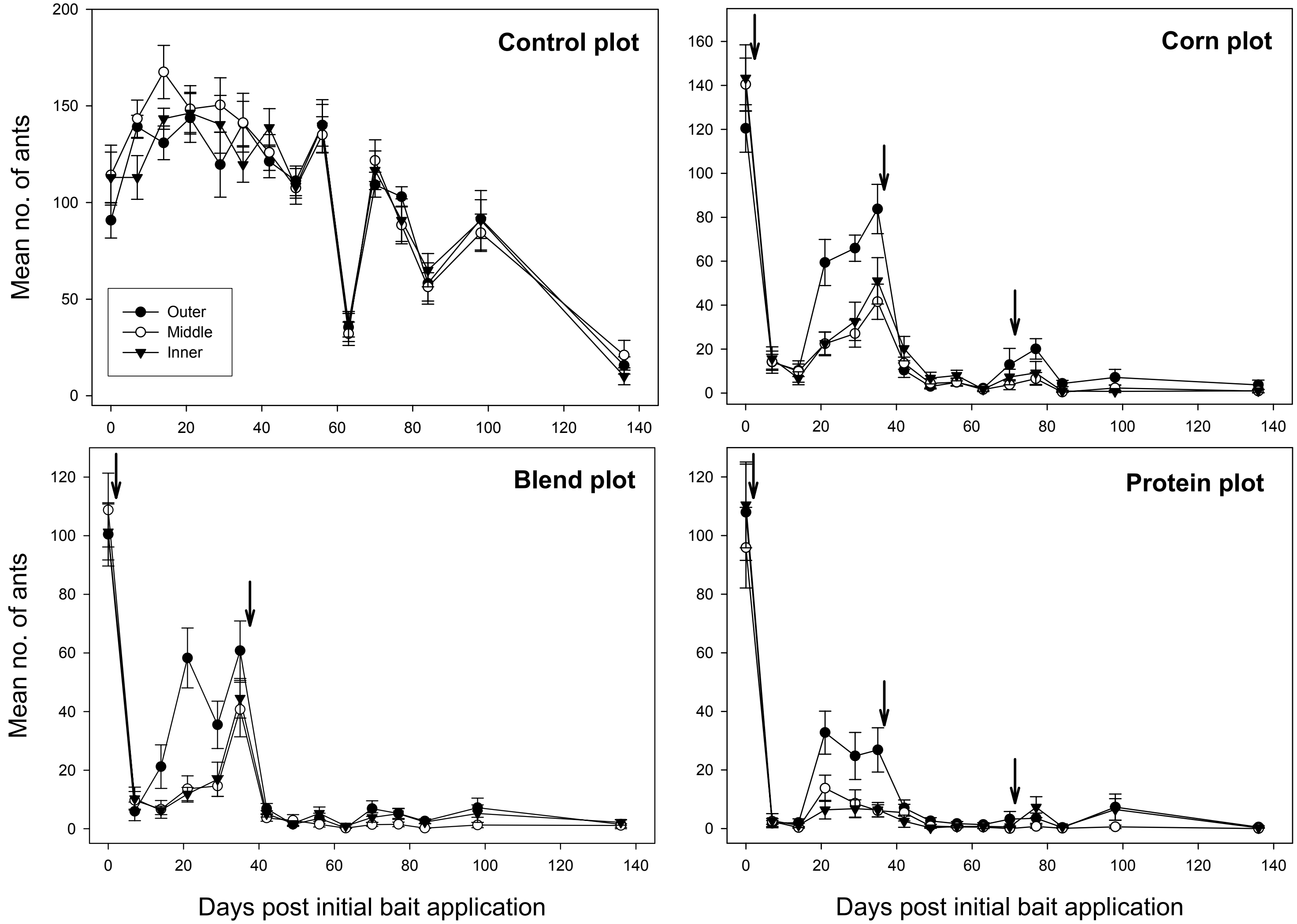

Figure 3. Mean numbers of ants (±1 SE) at three of the four bait card monitoring station types in each of the four plots during the course of the experiment. Arrows indicate timing of pesticidal ant bait applications. See Methods and Figure 1 for the relative positions of the three monitoring station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

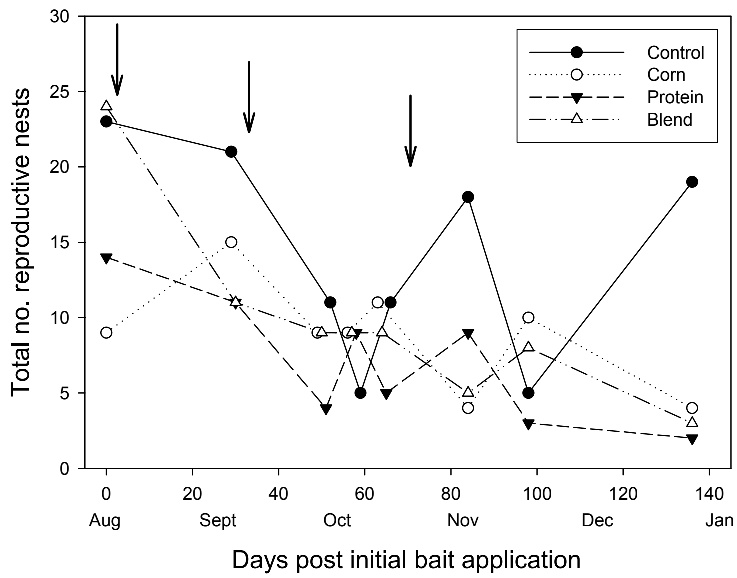

Figure 4. Total number of reproductive nests per monitoring quadrat in the four plots during the course of the experiment. Arrows as in Fig. 2.

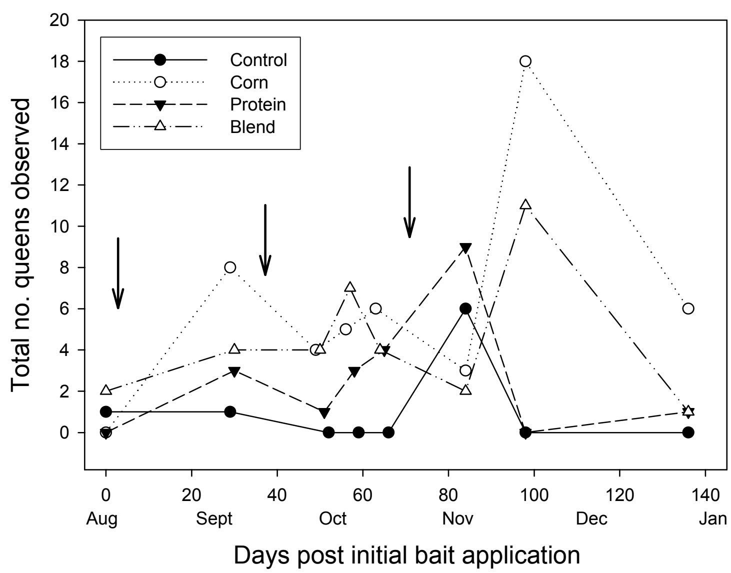

Figure 5. Total number of queens observed per monitoring quadrat in nest surveys in the four plots during the course of the experiment. Arrows as in Fig. 2.

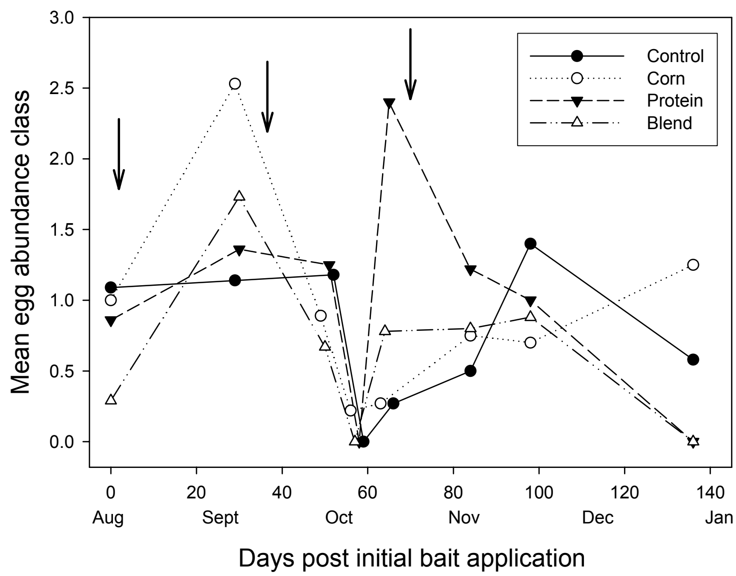

Figure 6. Mean egg abundance class of reproductive nests during nest surveys in the four plots during the course of the experiment. Arrows as in Fig. 2.

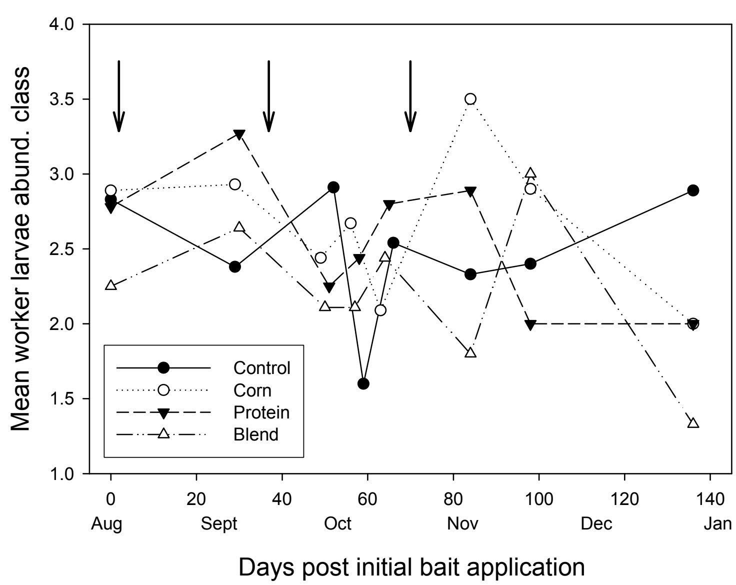

Figure 7. Mean worker larvae abundance class of reproductive nests during nest surveys in the four plots during the course of the experiment. Arrows as in Fig. 2.

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).

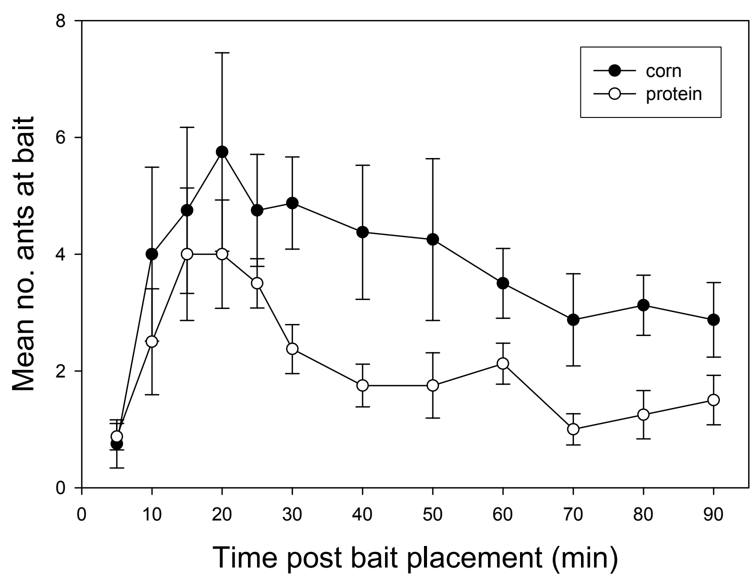

Figure 8. Numbers of ants attracted to the two granule types in 0.5 HP Ant Bait over the course of 90 minutes in side-by-side bait preference trials. Data shown are the means (± 1 SE) of eight replicate trials.

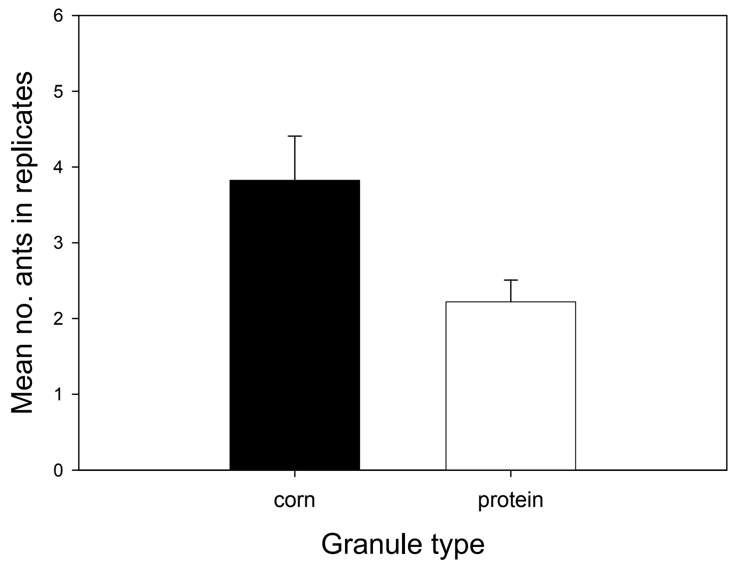

Figure 9. Numbers of ants attracted to the two granule types in 0.5 HP Ant Bait in side-by-side bait preference trials. Data shown are the means (± 1 SE) of the average counts of the eight replicate trials.

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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