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EVALUATION OF AN ANT BAIT AGAINST *LINEPITHEMA HUMILE* IN AUSTRALIA

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Abstract Argentine ant (Linepithema humile) has been present in Australia for approximately 100 years and has spread to many areas of temperate climate in southern Australia. Argentine ant has proven to be difficult to control under Australian conditions with the insecticidal products available up until now. During 2007 a new experimental ant bait containing both pyriproxyfen and hydramethylnon was tested against Argentine ant on the Mornington Peninsula in Victoria. The combination of the two active ingredients incorporated into a special matrix proved to be both very attractive to Argentine ant and effective in reducing populations over a ten week period during autumn. Further work is planned to investigate the effects of repeat treatments over a full spring-summer period.
Key Words Argentine ant, pyriproxyfen, hydramethylnon

INTRODUCTION

Argentine Ant (*Linepithema humile*) is native to South America but now established widely around the world, including Australia (Buczkowski and Bennett 2008, Suarez et al. 2001, Vogel et al. 2010). It was first recorded in Melbourne in 1939 and spread to many areas of temperate climate in southern Australia (Heterick et al., 2000; Rowles and O'Dowd, 2009; Walters, 2006) and has recently been found in Queensland (Suhr et al., 2009). Argentine ants are polydomous, unicolonial and form extensive supercolonies (Giraud et al., 2002, Klotz et al., 2008) and quickly out-compete native species (Holway, 1998; Human and Gordon, 1996) which makes them very successful invaders.

Control strategies based on liquid sprays and perimeter treatments typically provide short term reductions in activity because only foraging worker ants are removed (Rust et al. 2003). Baiting technology exploits many of the behavioural characteristics of the colony that promote contact between individuals including foraging activity, feeding behavior and tropholaxis, brood maintenance and grooming (Rust et al., 2003; Klotz et al., 2008). For Argentine ants, their unicolonial behaviour and high exchange rates between adjoining colonies (Markin, 1968) should also promote the wider distribution of the bait and toxin. However, the toxicant must also have amenable characteristics such as delayed toxicity and non-repellency.

The preferred foods of Argentine ants are honeydew and other insects (Klotz et al., 2008). As a result solid granular baits have met with little success in terms of eradication or long-term suppression (Baker et al., 1985; Cooper et al., 2008; Klotz et al., 2000; Krushelnycky and Reimer, 1998a; Kruschelnycky et al., 2004; Rust et al., 2003). Nevertheless, Argentine ants may be more amenable to solid granular bait during the early post-winter period when colonies are undergoing rapid growth and development and protein based baits in particular may be more attractive during this period of brood development (Rust et al., 2000). Other than urban infestations, where liquid baiting strategies may be more suitable, the most practical eradication or control measure remains broad-scale application of granular baits and so the development of more efficacious granular media and toxins remains an important goal (Rust et al. 2003).

Two active ingredients commonly used in granular baits are the juvenile hormone analogue pyriproxyfen and the ATP inhibitor hydramethylnon. Pyriproxyfen is most commonly used in baits designed for red imported fire ant which consist of reprocessed corn meal and soybean oil and may not be overly attractive to Argentine ants (Krushelnycky and Reimer, 1998a; Rust et al., 2003). Hydramethylnon has been utilized in both corn and protein based granules for a wide variety of ant species including Argentine Ant. However, control programs for Argentine ant have met with variable success (Baker et al., 1985; Klotz et al., 2000; Krushelnycky and Reimer, 1998a) because the toxin acted too quickly on foraging workers and failed to eliminate the queens and brood (Hooper-Bui and Rust, 2000; Klotz et al., 1996; Knight and Rust, 1991). The inability of foraging workers to first establish and then maintain foraging trails results in reduced exposure of the queens and brood to the toxicant (Rust et al., 2004).

Hooper-Bui and Rust (2000) evaluated mortality of workers and queens across a gradient of concentration of hydramethylnon in sugar-water. Queens appeared to be more sensitive to the presence of hydramethylnon and reduced consumption at all concentrations greater than ca. 0.04%. At the highest dose (1%) workers consumed 12 times more sugar-water per unit body weight than did queens. This suggests that bait formulations may not be optimized for queen mortality and that lower doses of active ingredient over a sustained period of time may be more effective in reducing queen numbers. Another possible reason for failure to eliminate queens proposed by Hooper-Bui and Rust (2000) is the concept of nutritional hierarchies among the queen caste.

As granular bait matrices have historical been configured for particular target species (eg. corn granules for *Solenopsis* spp.) few bait products have broad utility. Therefore a granular bait product which combines various possible food items maybe be more attractive to and efficacious on a wider range of pest ant species. Furthermore, food preferences can be seasonally and spatially variable and this has been clearly demonstrated for Argentine ants (Kruschelnycky and Reimer, 1998b; Rust et al., 2000). Therefore, even though a particular granule may appear be attractive to target species it may fail to control these species because the nutritional requirement in the nest at that time may be different. In this study I have attempted to combine the benefits of various food items combined into two different matrices but also two active ingredients with different modes of action at reduced loadings. This study forms part of an overall program of evaluation of this experimental ant bait against a wide range of pest ants.

Argentine ant is widespread on the Mornington Peninsula (Victoria, Australia) and present in high numbers within local recreation reserves. These reserves provided an ideal opportunity to evaluate the new experimental bait for control of Argentine ant. Various enhanced bait matrices and size fractions were offered to colonies of Argentine ant in the field to evaluate attractancy. One promising candidate (blend of two granules) was then used to evaluate efficacy across a wide range of sites.

MATERIALS AND METHODS

Bait Attractancy

On 15 February 2007, Argentine ants were offered a range of experimental blank baits based on corn or protein granules to evaluate preference for granule composition and size. Blank protein granules comprised of fishmeal and other food ingredients, and blank corn granules incorporating 15% soybean oil and other food ingredients were sieved to the desired size.

Various sieved fractions as well as a blend of the two granules were offered in a no-choice test. Bait stations comprised small A5 sized bait cards with a single 0.5 g sample on each. There were 5 replicates of each bait. Ant activity on bait cards was monitored for 4 hours from 12 noon until 4pm and time to total removal recorded. Weather conditions were fine and maximum temperature was 28°C. After 4 hrs all residual bait was retrieved and reweighed using a 2-place balance. For many bait types and replicates bait remained after 4 hours so an analysis of time taken to remove bait was not possible. However, weight of residual bait was analysed for statistical difference using a one-way ANOVA (Statistix v8).

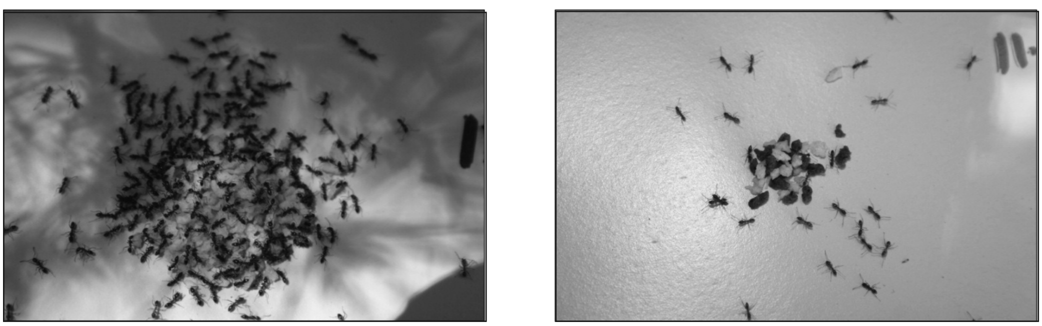

Figure 1. Argentine ants attending corn bait (a) and blended bait (b).

Bait Efficacy

Nineteen plots of varying size were selected based on the presence of moderate to high levels of infestation by Argentine ant. All plots were within Mornington Shire Council parks and reserves.

Eleven plots were treated with the experimental ant bait and varied in size from 2200 and 7600 m^2. The eight most heavily infested plots were treated at the target rate of 4 kg/ha and the remaining 3 at 2 kg/ha as a comparison. The eight remaining plots were used as controls but some had low levels of Argentine ants present. In each plot a core sampling area was used which comprised a central monitoring station and 4 others radiating out at 2.5 m intervals along the 4 compass points (Figure 2). Where space allowed, additional monitoring stations were placed at intervals along the north and south orientation out to 60 m from the central monitoring station to determine if the infestation was uniform. All control plots consisted only of the core area.

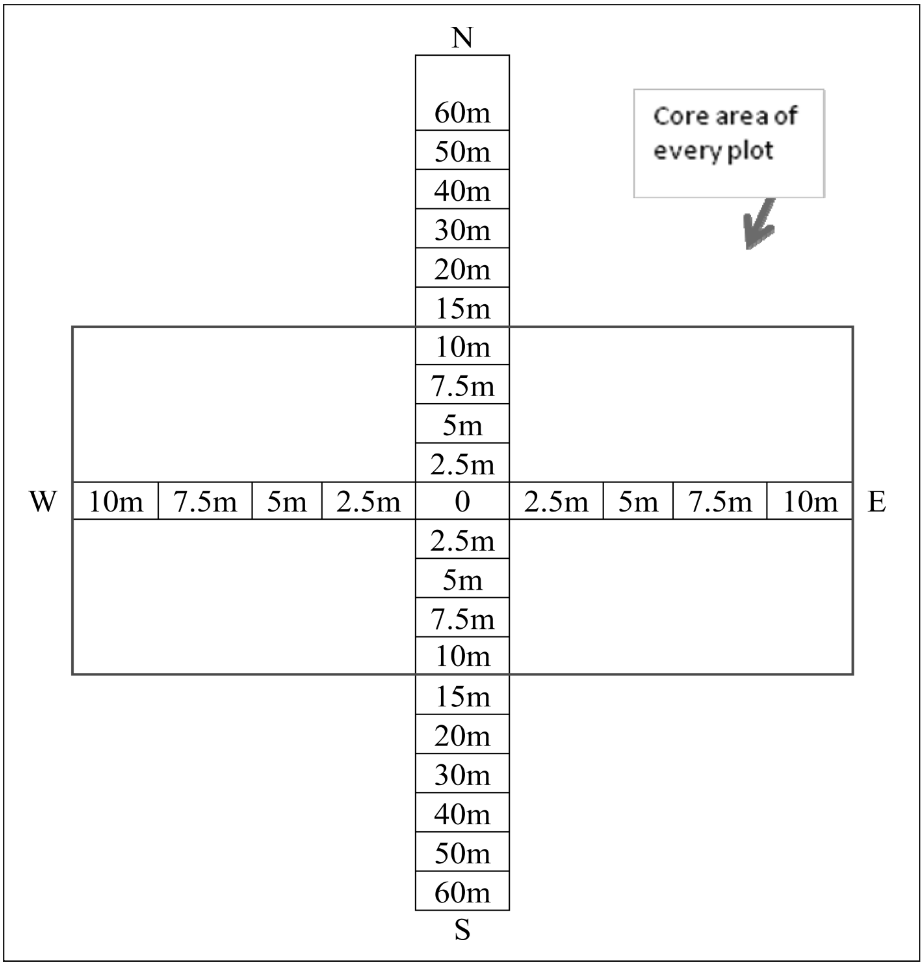

Figure 2. Standard plot layout.

Each monitoring station consisted of a small paper card on which a small amount of honey, egg mayonnaise and dog food was placed. Monitoring stations were checked at 30 minutes and 2 hours after placement and the number of ants present estimated according to the following rating system. Each recognizable ant taxa was rated separately according to a simplified abundance rating: 0 = No ants, 1 = 1-5 ants, 2 = 6-25 ants, 3 = 26-50 ants, 4 = 51-100 ants, 5 = 100+ ants.

Argentine ants were the dominant species in all plots representing ca. 88% of all ants at monitoring stations. However, a range of other species were present. These included: *Pheidole* spp. (*Pheidole* sp. gp. E and *Pheidole* sp. *hartmeyeri* gp.), *Crematogaster* sp. *queenslandica* gp., *Monomorium* spp. (*Monomorium* sp. *laeve* gp. and

Monomorium sp. *nigrius* gp.), *Iridomyrmex* sp.(black ants), *Rhytidoponera victoriae*, *Polyrachis* sp. and *Myrmecia* sp.. Ant samples were kindly identified by Dr Alan Anderson and Dr Ben Hoffmann of CSIRO Sustainable Ecosystems, Darwin.

The trial commenced on 5-6 March 2008 and pre-baiting assessments were conducted on all plots prior to bait application on the 11 treatment plots. Subsequent bait assessments occurred on 8-9 April 2008 (4 weeks) and 21-22 May 2008 (10 weeks). Daily temperature ranges for these three periods were 13.6-23.4°C, 14.2-25.3°C and 4.5-12.7°C respectively and no rain occurred within the previous few days of each assessment period.

Bait was applied during the late afternoon and evenings of 5 and 6 March 2008, avoiding the hot midday period. Bait was applied by hand broadcast utilising a gentle breeze to create a ca. 5 m swath width while traversing each block until all ground was covered with one pass in a north-south direction and another in a east-west direction.

Data Analysis

Data were analysed using the generalized AOV function in Statistix v. 8 (Analytical Software, Florida) and mean separation using Tukeys HSD all-pairwise comparison. Data was entered into the model as raw values and as log10 and SQRT transformed values. Log10 transformation provided the best fit for the model for Argentine ant abundance (Wilks Shapiro normality test, W = 0.95) whilst SQRT transformation provided a slightly better fit than Log10 transformation for total ant abundance (W = 0.95 vs 0.93 respectively). The key test for inclusion of any plot was that Argentine ant represented 50% or more of all ants at lures during the pre-treatment assessment. On this basis two of the 8 control plots were excluded. The three 2 kg/ha plots were also excluded from the analysis because of lower replication (3 only). These three plots were assessed on the same basis as the others but were only intended as a casual comparison.

RESULTS AND DISCUSSION

Bait Attractancy

As many bait stations still contained bait after 4 hours it was difficult to discern any patterns based on these criteria, certainly in terms of the protein granule (Figure 2). The unsieved protein granule varied in size and regularity of shape with some granules up to 7 mm long and clearly too large for Argentine ant to remove. However, there was little or no activity on the smaller fractions with the exception of the 0.5-1.0 mm fraction and ants were observed actively removing these smaller granules. Argentine ants seemed more interested in the corn granules and appeared to remove them faster than the protein granules. Size of the corn granules did not seem to be important, which may be related to their low mass.

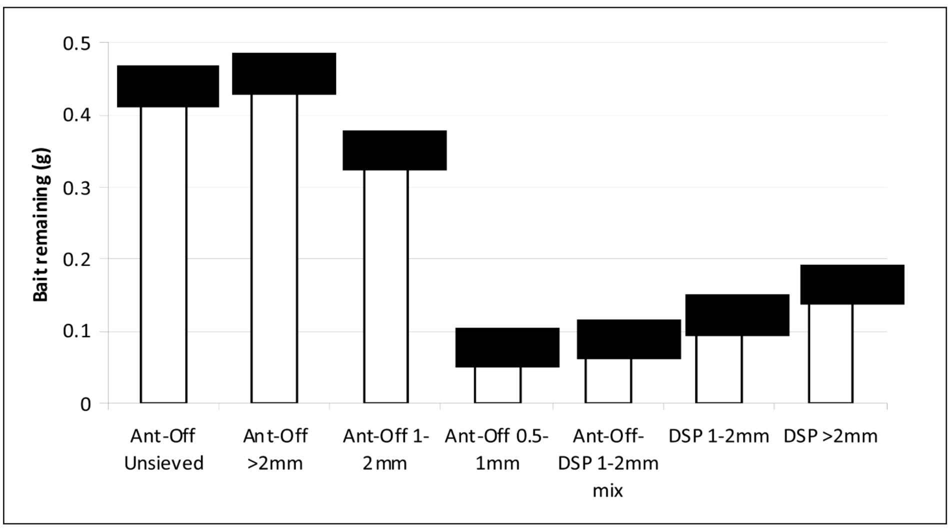

Figure 3. Mean Bait remaining (g) following exposure for 4 hrs. Above each bar is the time taken to total removal of all bait.

For remaining bait (after 4 hours) there was a clear and significant preference for corn granules over protein granules (P=0.0003). This was largely due to the difference between unsieved protein granules and the >2 mm fraction against all other baits. Ants were most active on the 0.5-1 mm protein fraction and the 1-2 mm blend of protein and corn granules. The preference for corn granules is somewhat surprising given that formulated corn-based baits designed for red imported fire ant have not proven to be attractive to Argentine ant elsewhere (Klotz et al., 2000; Kruschelnycky and Reimer, 1998b). However, as has been found with a number of other species, attractiveness has been improved by the addition of other food ingredients to the corn-based formulation (Webb, unpubl. data).

The apparent lack of overall attractiveness of the protein granule to Argentine ant in this study is a little puzzling given that other protein-based granular formulations appear to be attractive to Argentine ant elsewhere (Blachly and Forscher 1996, Forshler and Evans 1994a and b; Krushelnycky and Reimer, 1998a). However, the formulations are very different with commercial formulations based on ground up silkworm pupae which is a closer approximation to a natural insect diet; whereas the protein granule use in this study was a formulated mixture of human food ingredients. The smallest fraction (0.5-1 mm) of the protein mixture did appear to be the most attractive granule in this study and coincidently this granule size is the closest to that of the commercial silkworm –based granular products. Hooper-Bui et al. (2002) also determined a preference for food particle size in the range of 0.8-1.0 mm for Argentine ant and a range of other species.

Size of granule is significant to Argentine ants, although the larger corn granules were taken readily which suggests that weight is also a key factor. The blend of protein and corn was successful in terms of Argentine ant acceptance and removal, even though the 1-2 mm protein granules alone were not readily accepted. This suggests that once the foraging trail is established to the sample of bait then the generally all bait will be removed. This is consistent with trail establishment and recruitment of Argentine ants to clumped food sources (Silverman and Roulston, 2003).

Bait Efficacy

Prior to treatment, Argentine ants comprised the majority of ants attending monitoring stations at both the 30 min and 2 hr assessments (Figure 4). Among the other species the most abundant at lures were *Pheidole* spp. and *Crematogaster* sp. *queenslandica* gp. but this varied between plots.

Abundance at lures (total ants and Argentine ant) was consistently higher at the 2 hr counts than the 30 min counts (Figure 4) reflecting the time needed to establish full recruitment to the lure. The 30 min. and 2 hr counts were highly correlated (Pearsons co-efficient, r = 0.72 and r = 0.76 respectively) indicating the speed of recruitment was similar across all plots. Mean abundance rating for total ants and Argentine ant was similar (and very highly

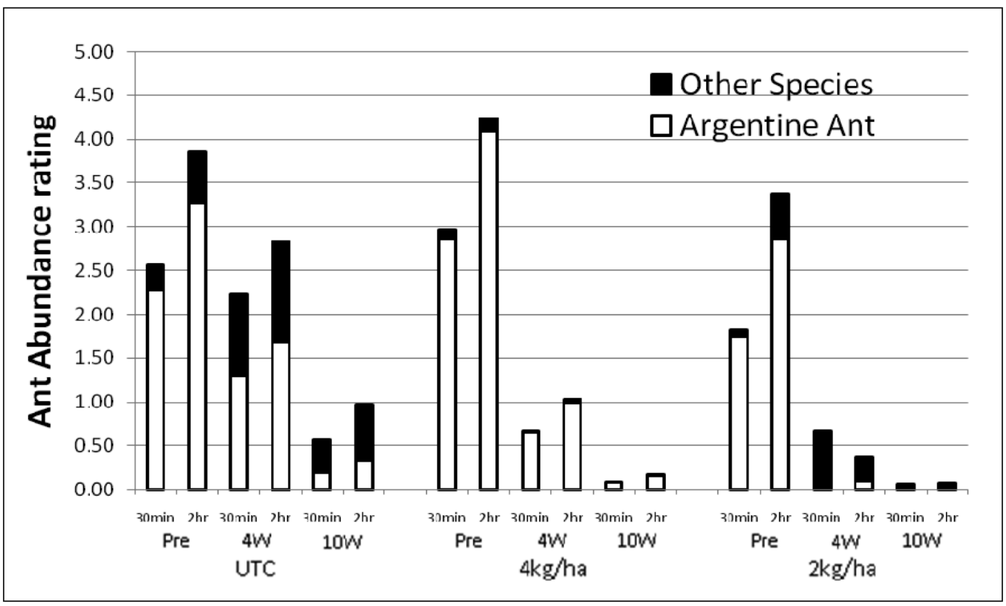

Figure 4. Ant abundance ratings for Argentine ant and other species (2 hour counts)

correlated) between the inner core plot and the whole plot for the 11 plots to be treated (Pearsons co-efficient, r = 0.95 and r = 0.97 respectively). Therefore detailed analysis will only consider the inner core plot for the 2 hr assessments.

The trial was conducted during late summer and autumn and the final assessment was in late May when daily temperature had declined significantly (from daily maximum of 25.5°C in April to 12.7°C in May. As a result ant activity was subdued, even in the control plots, as time went by. Colonies of Argentine ant are known to severely reduce foraging activity and contract in size as winter approaches (Klotz et al., 2008; Krushelnycky et al., 2005) and this decline was quite apparent here. Nevertheless, a treatment effect was evident. Both treatment and time were significant factors for abundance of total ants and Argentine ant (Total ants: Treatment $P < 0.001$, Time $P < 0.0001$; Argentine ant: Treatment $P < 0.05$, Time $P < 0.0001$). Clearly time was the most important factor in the decline in abundance of Argentine ant and this reinforces the notion that earlier timing of trial establishment is critical (Klotz et al., 2008).

While the number of foraging workers at monitoring stations declined rapidly, there was evidence that other species of ants continued foraging into the final month of the trial. Where Argentine ants had been dominant at monitoring stations previously, they were subsequently replaced by other species. This was not only evident from the overall proportion of Argentine ants relative to others species (Figure 4), but in at least two control plots Argentine ants were severely reduced in number at the 1 month assessment and virtually absent from monitoring stations in the final assessment, replaced by other species (largely *Rhytidoponera* and *Crematogaster*).

Pyriproxyfen has not previously been used to try and eradicate or control Argentine ant. However, hydramethylnon has been used in a number of studies using granular bait formulations. Using Maxforce granular ant bait (1% hydramethylnon), Krushelnycky and Reimer (1998a) successfully reduced foraging worker numbers in Haleakala National Park in Hawaii by 97% after 1 month with a single treatment but the most dramatic part of the decline occurred within 2 days. Ant counts at monitoring stations had recovered to 30% of original numbers within 1 month. A subsequent application reduced numbers again to 92% after 2 months and then numbers remained low. However, control numbers were also declining during this period, possibly linked to proximity to winter and brood decline. Despite success in lowering foraging worker numbers, a high proportion of nests remained active and this was attributed to a range of factors including rapid degradation of both the bait matrix and the active ingredient (which is UV sensitive) and lack of delayed toxicity, necessary to successfully impact the reproductive capacity of the colony.

Successful control of Argentine ant in urban environments (Forshler and Evans, 1994a and b; Blachly and Forshler, 1996) has been achieved using hydramethylnon-based granular baits in containerized systems. These systems appear to have the benefit of protection from UV light, and bait replenishment. Klotz et al. (2000) applied two granular formulations of hydramethylnon to confined urban spaces and achieved good control with the protein granule but not with the corn granule. In contrast, large scale field applications using broadcast application (this study, Kruschelnycky and Reimer, 1998a) may expose the granules to high UV exposure (and therefore toxicant degradation), and other environmental factors such as rainfall which may dissolve the granule or direct sunlight and heat which might dry out the granule, both of which might make the matrix unattractive or unpalatable. Vander Meer et al. (1982) determined a significant loss (55% within 10 hours) of active ingredient hydramethylnon from commercial Amdro Granular Ant Bait (0.88% hydramethylnon) when exposed to natural sunlight under experimental conditions.

In both laboratory and field studies, hydramethylnon appears to act rapidly on foraging workers and possibly too fast for sufficient active ingredient to be returned to the nest and affect queens and brood (Hooper-Bui and Rust, 2000; Klotz et al., 1996; Knight and Rust, 1991; Kruschelnycky and Reimer 1998a). In this study there were no sample points between pre-treatment and 1 month due, to logistic reasons. Therefore there is no way of knowing how quickly the reduction in numbers occurred. However, one could speculate that, at half the strength or less of standard hydramethylnon-based baits reduction in foraging ants should be slower. Whether this lowered concentration was also able to overcome the apparent sensitivity of queens (Hooper-Bui and Rust, 2001) could not be determined.

Unfortunately there are no studies to my knowledge on the effects of pyriproxyfen on Argentine ant and it was not the intent of this trial to attempt to separate the effects of the two active ingredients. Argentine ant is one of many target species for this experimental ant bait and for many species, the effects of pyriproxyfen have been well established (Klotz et al., 2008). Pyriproxyfen is also known to be relatively photostable (Sullivan and Goh, 1998) unlike hydramethylnon. Pyriproxyfen mimics natural juvenile hormone necessary for larval development and other biochemical processes related to embryogenesis and reproduction (Miyamoto et al., 1993). Its primary effect on ant colonies are reduction in fecundity and blocking of pupation (Glancey et al., 1990; Vinson and Robeau, 1974) leading to reduced worker replacement and colony collapse.

Despite the complications of the approaching winter and the lack of dominance by Argentine ant in some plots, the experimental ant bait was shown to be effective in significantly reducing numbers of foraging Argentine ants. However, Argentine ant was not eliminated and this mirrors results from Hawaii using the same bait (Krushelnycky pers. comm.). However, further work is warranted to determine if more effective control might be achieved by a program commencing earlier in the season and maybe also by multiple treatments. As Hooper-Bui and Rust (2001) speculated, mortality of queens may be enhanced by more constant exposure to lower levels of active ingredient.

One of the aims of this program was to develop a bait with broader acceptance by range of nuisance and invasive ants, including Argentine ant. So far, the experimental bait has shown promising results on a range of nuisance and invasive species, including Argentine Ant. Further work is ongoing.

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