# EVALUATION OF ANT BAITS FOR CONTROL OF PHEIDOLE MEGACEPHALA (HYMENOPTERA: FORMICIDAE) ON LORD HOWE ISLAND, AUSTRALIA

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Abstract Lord Howe Island is a small sub-tropical island off the east coast of Australia. African bigheaded ant *Pheidole megacephala* (F.) was discovered on the island in 2003 and been subject to ongoing attempts at eradication resulting from concerns over its impact on native biota. In 2006-2007 a trial was conducted to evaluate various commercial and experimental ant baits, including Amdro™ Granular Ant bait (hydramethylnon) and Distance® Plus Ant Bait (pyriproxyfen), for the control of *P. megacephala* on Lord Howe Island. Pyriproxyfen eliminated *P. megacephala* within 33 weeks, as did a combined pyriproxyfen/hydramethylnon experimental bait, whereas formulations containing hydramethylnon alone reduced numbers to low levels but did not entirely eliminate *P. megacephala* from those blocks. Abundance of *P. megacephala* in blocks treated with Hydramethylnon-based baits returned to normal by 91 weeks after treatment whereas abundance remained low in the block treated with pyriproxyfen.

Key words Pyriproxyfen, hydramethylnon, Lord How Island, African bigheaded Ant, Pheidole megacephala

### INTRODUCTION

Pheidole megacephala is a common tramp ant around the globe. It is present in many environments in Australia, the south Pacific and southeast Asia (Loope and Krushelnycky, 2007; Wetterer, 2007). This species is unicolonial, forms supercolonies is highly aggressive, and quickly dominates natural arthropod communities (Hoffmann, 1998; Wetterer, 2007). It was first recorded in Australia approximately 100 years ago but on Lord Howe Island in 2003. It is believed to have been introduced in soil imported before 2003 (Anonymous, 2007). While *P. megacephala* is a nuisance species in dwellings, there is concern for the ecological impact it may have on native biota (Anonymous, 2007).

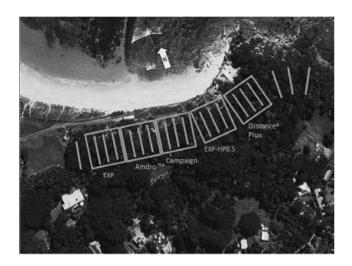
There are very few control options available for *P. megacephala* in Australia. There are several hydramethylnon-based granular baits registered for use on this species and more recently a pyriproxyfen based granular bait (Distance Plus) was approved for use on *P. megacephala* in Australia. Otherwise there are only a few consumer products that are unlikely to be viable options on a larger scale. *P. megacephala* is currently the target of an eradication campaign on the Lord Howe Island (Hoffmann, pers. comm.). This study was conducted to evaluate candidate products for control of *P. megacephala*.

### MATERIALS AND METHODS

## Distribution of *P. megacephala* in the Study Area.

Prior to establishing treatment areas, a survey was conducted to define the extent of the infestation of *P. megacephala* in the Ned's Beach area on Lord Howe Island. *P. megacephala* was abundant in

the narrow strip of coastal rainforest between the sea-cliffs and the adjoining private properties to the south (Figure 1) and extended into the adjacent private properties. The heaviest density was within the five blocks marked in Figure 1. A single transect was placed in the area adjacent to the carpark but density was relatively low and it was not suitable as an untreated control transect. Similarly, at the top end of the trail through the reserve, density was lower and in fact this proved to be the boundary of the infestation where *P. megacephala* co-existed with various other species including *Rhytidoponera victoriae* (Smith), *Paratrechina obscura* gp., *Paratrechina minutula* gp., *Tapinoma melanocephalum* (F.) and *Monomorium nigrium* gp. Three transects were placed in that area to monitor the interaction of the species present and hopefully detect any expansion of the infestation, but again they were considered unsuitable as untreated control transects.



**Figure 1.** Location of trial site at Ned's Beach. Each block contains three roughly parallel transects. Four transects are located outside of the designated blocks and are considered pseudo-controls only.

## **Trial Design**

Fourteen 50 m transects were marked out over the area of the apparent infestation. The walking track up the headland was the centre line through each transect. Each transect was perpendicular to the track and lures were positioned every 3 m either side of the track giving 10 lures per transect. At the end of each transect there was a ca. 10 m buffer to the edge of the apparent infested area (usually the cliff face on one side and a boundary fence on the other). After the pre-treatment assessment, five adjoining blocks of 50 x 50 m (0.25 ha) containing two transects each were marked out over the most seriously infested area, allowing for five different treatment blocks. The size of the infestation did not allow for replication because smaller treatment plots would be too small to eliminate the confounding effects of wide foraging territories which are common in species with large and co-operative colonies like P. megacephala (Warner et al., 2008; Vega and Rust, 2003). No control block was assigned due to low abundance or sporadic distribution of P. megacephala outside of the 5 blocks assigned active treatments. The transects placed either end of the five assigned blocks provided useful information on both temporal abundance of P. megacephala and their interaction with other species at the boundary of the infestation. These transects were not included in any statistical analysis. Later in the trial (week 9) a third transect was added to each block equidistant between the two existing transects to provide a better view of the distribution of *P. megacephala* within each block. This third transect in each block was excluded from statistical analysis to be consistent with previous assessments. However it showed that distribution of *P. megacephala* was relatively even across each of the blocks.

The results from four of the treated blocks are reported here. Block 1 (EXP) was treated with a new experimental ant bait. Block 2: Amdro<sup>™</sup> Granular Ant Bait (7.3g/kg hydramethylnon) manufactured by BASF. Block 3: Campaign Ant Bait (7.3g/kg hydramethylnon) manufactured by Sumitomo Chemical. Block 4: EXP-HP0.5 (2.5g/kg pyriproxyfen plus 3.65g/kg hydramethylnon, Sumitomo Chemical). Block 5: Distance Plus Ant Bait (5g/kg pyriproxyfen, Sumitomo Chemical).

At the time of the trial, Amdro (along with some other equivalent hydramethylnon-based baits) was the only bait registered for use on *P. megacephala* in Australia. Distance Plus has subsequently been registered for this purpose based partly on the results of this trial. Both Campaign and EXP-HP0.5 are experimental baits and remain under development for use in Australia. They both currently have approved uses under special permit in Australia in government-sponsored eradication programs for yellow crazy ant (*Anoplolepis gracilipes*) and little fire ant (*Wasmannia auropunctata*).

Amdro and Campaign were applied at the currently approved Amdro rate of 2.5 kg/ha. Distance Plus was applied at the now approved rate of 2 kg/ha as was EXP-HP0.5, for which 2 kg/ha is the proposed use rate.

Bait was broadcast by hand evenly over the treatment area using a swath width of 5 m and travelling in two opposite directions and covering the area twice with bait. Bait was applied twice, first on 13 March 2006 and again on 5 April 2006 (3 weeks later). The decision to treat twice was based on the very high density of *P. megacephala* and the need to ensure that adequate bait reached all nests.

Ant abundance was assessed using lures comprising honey and tinned fish (ca. 1-2 g of each) placed on a small 8 x 6 cm sheet of paper at each designated point on each transect. Due to the large number of ants present at the lures, counting was not feasible. Ant abundance was assessed at 10 minutes and 1 hour after placement using the following rating system: 0 = no ants, 1 = 1-5 ants, 2 = 6-25 ants, 3 = 26-50 ants, 4 = 51-100 ants and 5 = >100 ants. This rating system has been used previously in similar high density situations (Webb, 2011; Webb and Hoffmann, 2013). Recruitment to lures prior to treatment (utilizing all lures in the five blocks plus the four additional untreated transects) was rapid with almost full recruitment achieved within 10 minutes. Abundance ratings at the two assessment times were highly correlated prior to treatment (Pearsons correlation R=0.65, P<0.001) and this did not change following treatment. Hence only the 1 hr assessments are reported here.

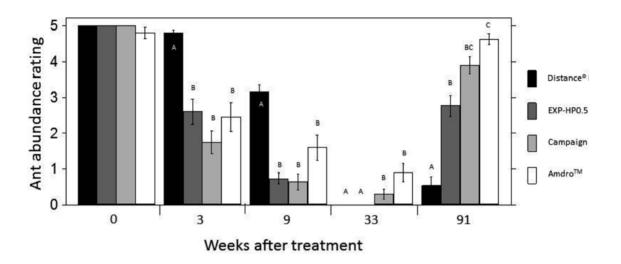
Ant samples were identified by Dr. Ben Hoffmann of CSIRO Sustainable Ecosystems, Darwin. **Statistical Analysis** 

As 5 treatment regimes were superimposed over the core infestation, the four untreated transects established on the periphery of the infestation could not be considered untreated controls. Statistical analysis was limited to comparisons between treated blocks within each sample period using all 20 lures as replicates. The third transect at week 9 was not included in the analysis. Non-parametric Kruskal-Wallis one-way ANOVA (Statistix ver. 9, Analytical Software) was used to compare treatments.

## **RESULTS**

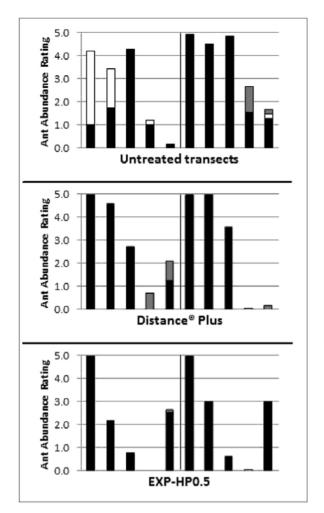
All three baits containing hydramethylnon caused a decline in abundance of ants attending lures within 3 weeks, whereas the first evidence of decline in the Distance Plus block was at 9 weeks after first treatment (Figure 2). However, at 33 weeks after treatment, no ants were present at lures in the Distance Plus and EXP-HP0.5 blocks while ants still remained in the Campaign and Amdro™ blocks (ratings of 0.4 and 1.0 respectively). As there were no appropriate control transects available it is not possible to be certain that the declines in abundance in the treated blocks were entirely due to baiting. However, *P. megacephala* remained present in the untreated transects throughout the trial (see Figure

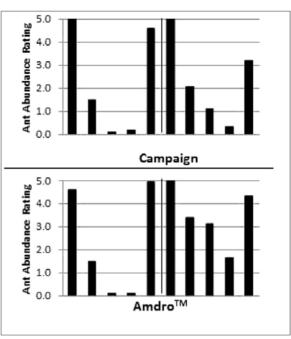
3) and abundance in all treated blocks, with the exception of Distance Plus, returned to close to pretreatment abundance by week 91 (Figure 2). The decline in abundance from week 9 to week 33 in the untreated transects (Figure 3) is difficult to explain. It is possible that some unknown environmental factor caused a general decline in numbers during winter (between weeks 9 and 33) but there were no obvious perturbations in rainfall or temperature. It is also possible that baiting in the blocks adjoining the untreated transects caused a broader decline in abundance.



**Figure 2.** Changes in *Pheidole megacephala* abundance following baiting. For each time period bars with the same letter are no significantly different using Kruskal Wallis non-parametric ANOVA. Error bars are calculated using raw data.

Of the three untreated transects at the top of the headland, the most dramatic decline in *P. megacephala* occurred in the transect most adjacent to the Distance Plus block (mean rating of 4.86 at week 9 declining to 0.6 at week 33 and finally zero at week 91). All blocks adjoined heavily infested semirural private property and it is likely that re-establishment occurred over the period of the study. This is indicated when data for each side of the walking track are considered separately (Figure 3). In the untreated transects, abundance of *P. megacephala* was higher on the RHS of the track adjoining the private property, as it was also for the Amdro, Campaign and EXP-HP0.5 treated blocks. This appeared not to be the case for the Distance Plus block. On both sides of the track in the Distance Plus block abundance of *P. megacephala* was reduced to zero by November 2006. However, no rebound in abundance was evident on the RHS at the December 2007 assessment whereas a small rebound occurred on the LHS (Figure 3). This was the result of a small pocket of infestation on the left hand side of one transect but the other 2 transects remained clear of ants. It is not evident whether this was a focal point for recovery of an existing colony or a new introduction. Aside from one lure in the EXP-HP0.5 block where *Paratrechina* sp. occurred at week 91, the block treated with Distance Plus was also the only block where species other than *P. megacephala* occurred during later assessments, mostly Paratrechina spp. These were only detected in the block from week 33 onwards. The pyriproxyfen treated block adjoined the area at the top of the headland where other species co-existed with P. megacephala.





**Figure 3.** Abundance ratings for *Pheidole megacephala* in black, *Rhytidoponera victoriae* in white, various ant species in grey. 5 bars for left and right represent assessment: pre, 3 wks, 9 wks, 33 wks, 91 wks, for left and right-hand sides of main track.

### DISCUSSION

All treatments substantially reduced abundance of foraging *P. megacephala* at lures over time. The reduction in numbers in the Amdro, Campaign and EXP-HP0.5 treated blocks was more rapid than for Distance Plus, which was to be expected given the nature of the two active ingredients. Amdro and Campaign did not achieve full elimination from lures at any point in time, and by the end of the study abundance had returned to near pre-treatment levels. While the decline in abundance in the EXP-HP0.5 block generally mirrored that of the Amdro and Campaign blocks, EXP-HP0.5 achieved zero abundance at lures by November 2006 (week 33) as did Distance Plus. At the final assessment, 12 months later, abundance in all treated blocks, with the exception of Distance Plus, had returned to close to pre-treatment levels. Abundance in the Distance Plus block remained at less than 20% of pre-treatment levels and largely this resulted from one small patch within the block and not on the boundary. The rest of the lures were either free of ants or occupied by a range of other ant species.

Amdro and other hydramethylnon-based baits have commonly been used to control or eradicate P. megacephala and other invasive ants (Apperson et al., 1984; Causton et al., 2005; Hoffmann, 2011; Hoffmann et al., 2011; Hoffmann and O'Connor, 2004; Klotz et al., 2000; Krushelnycky and Reimer, 1998). Amdro has been used in the eradication campaign on Lord Howe Island (Hoffmann, pers. comm.). There is a need for a wider range of options because hydramethylnon is unstable in ultraviolet (UV) light. Unless bait is harvested by ants rapidly, the active ingredient can be degraded under UV exposure (Chakraborty et al., 1993; Malipudi et al., 1986; Vandermeer et al., 1982). The speed of action may be too rapid to ensure thorough colony integration of the active ingredient, allowing colony resurgence (Hooper-Bui and Rust, 2000; Klotz et al., 1996; Knight and Rust, 1991; Kruschelnycky and Reimer, 1998; Oi et al., 1994, 2000). Ant baits utilizing slow acting insect growth regulators such as methoprene, fenoxycarb and pyriproxyfen have been successfully used in control programs against Solenopsis invicta Buren in the U.S., Australia, Taiwan (Hwang, 2009; Vanderwoude et al., 2003; Williams et al., 2001) and have reproductive and developmental effects on P. megacephala (Banks and Lofgren, 1991; Edwards, 1975; Glancey et al., 1990; Hsieh and Su, 2000; Lim and Lee, 2005; Reimer et al., 1991; Reimer and Beardsley, 1990; Vail and Williams, 1995). In recent times, pyriproxyfen granular bait has been used in the field against *P. megacephala* and *Monomorium destructor* (Jerdon) in Australia (Webb and Hoffmann, 2013) and Wasmannia auropunctata Roger in Hawaii (Souza et al., 2008). Pyriproxyfen has also been shown to be stable under normal environmental conditions (Sullivan and Goh, 2008; Webb et al., 2011) and a good candidate for effective residual control.

Webb and Hoffmann (2013) reported on the results of 4 field trials in northern Australia using Distance Plus against *P. megacephala*. In all cases there was a decline in abundance of worker ants at lures over a period of ca. 3 months. Although there was a large gap in assessment between 9 and 33 weeks in this trial (coincident with winter), there was a dramatic decline in abundance between these two assessments. It is possible that reduction occurred closer to the 9 week assessment than the 33 week assessment.

In all of the 3 blocks treated with hydramethylnon-based baits, abundance had returned to close to pre-treatment levels by the final assessment (ca. 18 months after initial treatment). It is not clear whether this represents resurgence of existing colonies which were not completely eliminated or the result of re-infestation from the adjoining private properties, or both. Persistently higher abundance adjacent to private property suggests that re-invasion has occurred. Abundance in the Distance Plus block remained low even after 18 months and despite adjoining infested private property. Similar effects were evident for S. invicta in Texas where abundance increased more dramatically in the plots treated with Amdro alone than in the Esteem Ant Bait (5g/kg pyriproxyfen) only plots (Drees et al., 2005). Collins et al. (1992) suggested that reduced resurgence in abundance of Solenopsis invicta with fenoxycarb-based bait relative to hydramethylnon-based bait may be the result of retention of the insect growth regulator (IGR) within the colony particularly by the longer lived major workers, which continue to transfer the active ingredient to any remaining reproductives and brood, or to newly adopted queens. This might also be exacerbated by the propensity of IGR (in this case methoprene) to induce higher production of major workers (Pheidole bicarinata Mayr) (Wheeler and Nijhout, 1983). An alternate theory is that the persistence of these major workers in IGR-treated areas long after reproductive capacity is lost may still prevent the establishment of new incursions through active interference (Barr, 2002; Barr et al., 2002).

A formulated blend of hydramethylnon and pyriproxyfen (EXP-HP0.5) did not appear to provide the expected blending of the biological activity of the two active ingredients.

Although zero abundance was achieved at 33 weeks, the same as pyriproxyfen treated block, by 91 weeks abundance had returned close to pre-treatment levels, although still lower than both hydramethylon-alone treatments. Similar results have been obtained for *S. invicta* in the U.S. using either formulated blends of hydramethylnon and IGRs, or blends of the two individual products (Barr and Best, 2002; Drees and Calixto, 2010; Drees et al., 2005). It is possible that the lower loadings of each active ingredient may be below the threshold for effective sustained control by one or other of the active ingredients. The balance between attractancy and repellency is delicate because it is known that formulated mixtures utilizing full dose levels of the two active ingredients (ie. 5 g/kg pyriproxyfen and 7.3 g/kg hydramethylnon) are repellent to some ant species (Webb, unpubl. data). Repellency was evident for yellow crazy ant and Argentine ant (*Linopithema humilis* (Mayr)) but not for *P. megacephala* or tropical fire ant (*Solenopsis geminata* (F.)). Similarly, for *S. invicta*, Barr et al. (2002) found no difference between full dose and half dose blends of S-methoprene and hydramethylnon.

Application of IGR-based ant baits may have an effect beyond the treatment boundary through resource sharing in some species. Oi et al. (2000) suggested that the delayed action of pyriproxyfen on pharoah ant (*Monomorium pharaonis* (L.)) relative to hydramethylnon may facilitate the wider distribution of the toxin among adjoining colonies through resource sharing and trophyllaxis. Using dye stained pyriproxyfen bait, Vail et al. (1996) showed contamination of *M. pharaonis* in untreated regions of an apartment block indicating interaction between widely dispersed colonies presumably involving resource sharing. Drees et al. (1992) found that spot treatment of *S. invicta* mounds with fenoxycarb resulted in reduction in mounds 7 m away. In this study there appeared to be reduced abundance of *P. megacephala* in an untreated area immediately adjacent to the Distance Plus block which may be evidence of this phenomenon.

Distance Plus has been shown to be effective on *P. megacephala*. There appeared to be some benefit in combining hydramethylnon and pyriproxyfen in a single bait for control of *P. megacephala*. The combined bait provided effective control mid-term, similar to pyriproxyfen alone, but did not prevent resurgence entirely, although more so than hydramethylnon-only bait formulations.

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