COMPARATIVE ATTRACTIVENESS OF TWO PYRIPROXYFEN-BASED ANT BAITS (DISTANCE® AND DISTANCE® PLUS) TO INVASIVE AND NUISANCE ANTS IN AUSTRALIA

G.A. Webb
Sumitomo Chemical Australia, Epping, NSW, 2121, Australia
Email: gawebb@sumitomo-chem.com.au

Summary

Nutritional requirements vary between ant species and the composition of ant baits designed to eliminate colonies of invasive and nuisance ant species needs to take this into account. Simple corn and oil-based ant baits have been effective for only a limited range of species. Distance® Ant Bait (5 g/kg pyriproxyfen) has been very effective in the Australian eradication program for red imported fire ant (*Solenopsis invicta*) but is less attractive to a wide range of other important pest species. Modifications of this standard formulation were tested on many different species and one particular formulation, Distance® Plus Ant Bait (also 5 g/kg pyriproxyfen), proved to be attractive to a wide range of species. Data from many choice and no-choice tests directly comparing the two formulations are included here. The incremental advantage in attractiveness of Distance® Plus over Distance® was evident for many species particularly those species that have a higher preference for carbohydrate and protein than lipids. These included yellow crazy ant (*Anoplolepis gracilipes*), green tree ant (*Oecophylla smaragdina*) and pale tyrant ant (*Iridomyrmex pallidus*). There was also a general improvement in bait attractiveness for some other species, including those for which Distance® was considered suitable for control.

Keywords: ants, invasive ants, ant bait, pyriproxyfen, attractiveness

INTRODUCTION

Ant baits based on pyriproxyfen and other juvenile hormone mimics have been used extensively for the control of red imported fire ant in various parts of the world (Hwang 2009, Vanderwoude *et al.* 2003, Williams *et al*. 2001). These baits are based on a simple formulation of reprocessed corn grit, and refined soybean oil containing the active ingredient, and largely result from the work of Lofgren and colleagues some 50 years ago (Lofgren *et al.* 1961, 1964, Jouvenez *et al.* 1974). Their work showed that corn based granular bait using refined soybean oil as the attractant was suitable for red imported fire ant. However, it is known that even in red imported fire ant colonies the nutritional requirements are complex, requiring also carbohydrate and protein at various stages of colony development (Vinson 1968, Sorensen and Vinson 1981, Sorensen *et al.* 1983, Stein *et al.* 1990). Similar nutritional complexity has also been demonstrated for some other species as well (Edwards and Abraham 1990, Sanders *et al.* 1992, Haack *et al.* 1995, Kay 2004, Loke and Lee 2004, 2006, Norasmah *et al.* 2006).

Existing corn-based baits are also known to be attractive to African bigheaded ant (*Pheidole megacephala)*, *Monomorium* spp. and little fire ant (*Wasmannia auropunctata)* (Vail and Williams 1995, Vail *et al.* 1996, Taniguchi *et al.* 2003, Causton *et al.* 2005, Sousa *et al.* 2008). However, attempts to control other species with such baits has met with less success and for some species the simple corn and oil formulations are less attractive than protein-based matrices (Bennett *et al.* 2013, Klotz *et al.* 2000, Rey and Espadaler 2004, Stanley 2004, Stanley and Robinson 2007). These species typically have a higher dependence on protein (from insects) and honeydew in their diet and therefore require bait products to supply alternative sources of protein and carbohydrate to be attractive.

Over the past 10 years, various modifications to the standard corn and oil formulation in Distance® have been investigated to improve attractiveness for a wider range of ant species than just the original key target species, red imported fire ant. One particular modification proved to be attractive to a number of different species including some which normally have higher dependence on carbohydrate and protein. This formulation has subsequently been approved for use in Australia as Distance® Plus and is labelled for a wide range of species. Distance® Plus has recently been shown to be effective on African bigheaded ant, yellow crazy ant and Singapore ant (*Monomorium destructor*) (Webb and Hoffmann 2013, Webb 2014). As part of the development program for Distance® Plus a large number of trials were conducted in Australia and some other locations to assess the relative attractiveness of Distance ® and Distance® Plus to a range of invasive and nuisance ant species. These trials are reported here.

MATERIALS AND METHODS

Thirty (30) separate trials are included here. In two trials, two species were dominant and so Table 1 shows 32 trials. These trials range across many different ant species and vary in execution but common to all is the comparison of attractiveness of two ant bait formulations, Distance® and Distance® Plus. Distance® is the standard corn grit with

adsorbed soybean oil containing the active ingredient. Distance® Plus has the same basic formulation but with the addition of a proprietary mix of human food grade ingredients. Species present in these trials are red imported fire ant, tropical fire ant (*Solenopsis geminata*), African bigheaded ant, Singapore ant, greenhead ant (*Rhytidoponera victoriae*), yellow crazy ant, meat ant (*Iridomyrmex* spp.), green tree ant, black ant (*Iridomyrmex* spp.) and pale tyrant ant.

Due to the large number of trials described here, only the key characteristics of the design and execution of each of the trials are provided in Table 1. Variations in assessment methodology are also shown in Figure 1. Both choice and no-choice tests were conducted but not in all locations or for all species. In both choice and no-choice tests bait samples were placed at relevant spacings which reflected colony density with the intention that each replicate represented a discrete colony or group of colonies. Where nests were small and cryptic and/or ant density very high; for instance with yellow crazy ant on Christmas Island and at Nhulunbuy it was not possible to be certain that each replicate serviced different colonies. In choice tests, colonies were not only allowed to choose, but also to utilise both baits once the bait was discovered. In many cases ants clearly discriminated against one bait but then removed it once the preferred bait was taken. There were very few cases where the non-preferred bait was completely avoided. Bait was offered generally on small plastic or paper cards (ca. 10 x 10 cm), in small disposable plastic sample cups (25mL) or on the lids from these cups, or in plastic 5mL weighboats or in one case directly onto the ground (Figure 1). In one trial on green tree ant larger capacity weighboats were used to contain the 10 g bait samples offered. Where the cup or weighboat method was used remaining bait was physically weighed and when placed on bait cards or directly on the ground, bait remaining was estimated. Generally bait cards, cups and weighboats were placed near active nests or foraging trails and positioned such that the chance of discovery was roughly equal. For choice tests on green tree ant using weighboats positioned in trees, separate weighboats were positioned side by side rather than placing both baits in one weighboat.

Various measures of bait attractiveness were used. Bait removal was assessed either by weighing the remaining bait, or estimating the proportion remaining (%) generally assessed in increments of 10%. The amount of bait placed in each trial varied from 0.5 g through to 10 g and was largely dependent on the length of time available for the study. Most trials were conducted using only 0.5-1.0 g of bait per sample. Time to total bait removal was also a common metric. The time allowed in each trial varied from 45 minutes through to 5 days and was largely set by logistic constraints on time and interruptions by weather conditions (rainfall and high temperatures). In many trials not all bait samples were completely removed within the time frame of the trial and hence many mean values were recorded as greater than (>) the trial time period. Logically, such values should be considered underestimates of the total time required for ants to remove the bait and this metric is considered relatively insensitive unless all bait is removed. In trials where not all bait was removed during the trial period the data is presented only as means foreach bait and no statistical analysis was applied. In two trials, Brisbane (April 2005) and Katherine (October 2004), more than one ant species was present at bait stations. In all other trials only the ant species specified was present at bait stations or almost completely dominated the bait stations. In Brisbane 10 replicates in total were included in the trial of which 6 were dominated by red imported fire ant and two were dominated by greenhead ant. Of the remaining two, black ants (*Iridomyrmex* spp.) solely attended one bait station and all three species were present in low numbers at the other. These final two replicates are not considered further here. With the exception of this final replicate (with all three species present), once recruitment to the bait had occurred the bait station was then eventually dominated by that species. Separate analyses were conducted on the 6 replicates dominated by red imported fire ant and the two dominated by greenhead ant. In Katherine, four of the total of 9 bait stations in that location were dominated by African bigheaded ant and five by Singapore ant and for the purpose of analysis these are effectively considered different trials. With the exception of the corn field site in Brisbane, all other sites used for bait attractiveness trials were believed to be unaffected by any previous ant control activities. The site in Brisbane had been treated three weeks previously with Distance® by Biosecurity Queensland as part of the government eradication program. Access to untreated colonies of red imported fire ant is largely not possible given that infestations are normally treated as soon as they are discovered. Hence the results from this trial location for both red imported fire ant and greenhead ant may have been compromised by the effect of the previous treatment in terms of their foraging ability, but this is not likely to be relevant for a comparative assessment such as this. Further, pyriproxyfen is slow-acting and its primary effect is on the reproductive capacity of the colony, not directly on foraging worker ants.

In humid tropical locations such as those in northern Australia, ant bait will likely absorb moisture from the air. Moisture absorption was tested in a number of trials in these locations and was generally found to be around 5% or less for both products and therefore not likely to significantly impact the results of these trials. These moisture absorption data are therefore not presented here. In a few trials, the mean measured amount of Distance® remaining actually exceeded the nominal starting weight and this is the result of moisture absorption co-incident with minimal or no bait removal.

Trials were generally conducted either in the early morning or in the afternoon in an attempt to utilise the optimal foraging time. This was particularly so for trials conducted in northern Australia where ant activity is subdued during the middle of the day. In southern Australia, the winter period was avoided because ant foraging is significantly reduced during this period. However, two trials on meat ant were conducted in May and early June and both were delayed till the middle of the day when ant foraging activity increased.

Ants collected during almost all of the trials were kindly identified by Drs Ben Hoffmann and Alan Andersen (CSIRO Tropical Ecosystems, Darwin). In the dragonfruit trial in Darwin, three species of unidentified small black *Iridomyrmex* were present: *Iridomyrmex* sp. A (*gracilis* gp) with other minor species including *Iridomyrmex* ANA sp. 4 and *Iridomyrmex* ANA sp. 3 (*mattiroloi* gp), all referenced back to the CSIRO Darwin ant collection.

Data in all trials was checked for normality using Wilkes Shapiro test (Statistix ver. 10, Analytical Software, Tallahassee, Florida). Where data was considered normally distributed or could be transformed to achieve normality, simple t-tests were used to discern any statistical differences in bait preference between Distance® and Distance® Plus - paired t-test for choice experiments and two sample t-test for no choice experiments. Data based on percentage remaining bait was transformed using an arcsin transformation. In many of the trials, there were large differences in bait removal between Distance® and Distance® Plus – often Distance® Plus was completely or almost completely removed from all replicates whereas the opposite was true of Distance®. Such data could not be adequately transformed. Alternative non-parametric techniques such as Wilcoxon rank sum test and Wilcoxon signed rank test could not be used in many cases because there were so few untied pairs. Therefore, analysis values are shown in Table 1 only where data used in t-tests was normally distributed with or without transformation.

Figure 1: Various methods for attracting ants to bait used in trials. A. African bigheaded ant in no-choice test on plastic card. B. Yellow crazy ant in choice test on plastic card, C. Singapore ant in plastic weighboat, D. Tropical fire ant in plastic sample cup, E. African bigheaded ant on ground placement, F. Green tree ant weighboat pinned to tree branch.

RESULTS

Red Imported Fire Ant (*Solenopsis invicta*)

Bait preference of red imported fire ant was tested in a choice test at only one site. Bait removal was relatively slow, indicated by the fact that for some replicates bait remained at the end of the trial. However, there was a significant difference in the mean amount of bait remaining on bait cards after 5 hours ($T = 3.56$, $p < 0.05$) indicating that Distance® Plus was preferred over Distance®.

Tropical Fire Ant

Three bait preference trials (all no-choice tests) were conducted on tropical fire ant in Darwin. In the first two trials, all Distance® Plus was removed but Distance® remained at some bait stations at the end of the trial (Table 1). No statistical analysis of either bait remaining or time to total removal was possible. In trial 1, bait remained in three of the Distance® bait cups (20, 20 and 40% respectively) but had been completely removed from the other two and all of the Distance® Plus bait cups. The mean removal time for Distance® Plus was 53 minutes. In the second trial, bait remained on just two of the Distance® bait cards (60 and 80% respectively) but had been completely removed from the other three and all of the Distance® Plus bait cards. The mean removal time for Distance® Plus was 49 minutes. In the 3rd trial there were similar mean amounts of both Distance® and Distance® Plus remaining (0.31g and 0.33g respectively) and bait remained on most bait cards at the end of the trial.

African Bigheaded Ant

Three choice tests were conducted in the Northern Territory in 2004-2005 and a no-choice test was conducted on Lord Howe Island in 2006 (Table 1). In the trial on a suburban property in Katherine there was no difference in the mean amount of bait remaining ($T=1.6$, $p>0.05$). At only one of the 4 replicates was substantial bait removed (100% for Distance® Plus and 84% for Distance®). It was noticeable that large numbers of ants attended the bait cards and with the exception of one replicate, where all Distance® Plus and most Distance® was removed, ants appeared to be more intent on imbibing oil from granules than physically removing granules. In trial 2, in a jackfruit plantation near Darwin, all bait was removed from bait cards and the speed of removal of Distance® Plus was significantly faster than Distance® (mean of 76 minutes vs 109 minutes respectively) ($T=3.8$, $p<0.05$). On a suburban property in Darwin (trial 3), Distance® remained on all five bait cards (mean of 0.66 g) but the 1 g samples of Distance® Plus were completely removed from all but two of these (mean of 0.12 g). Significantly more Distance® Plus was removed overall ($T=5.09$, $p<0.01$). The no-choice test on Lord Howe Island showed no difference between the two formulations for remaining bait ($T=0.83$, $p>0.05$). Bait remained at two of the four Distance® bait placements and just one of the Distance Plus® placements.

Singapore Ant

A single choice test was conducted in Katherine in 2004 in conjunction with the trial on African bigheaded ant on the same property (Table 1). The two species were co-dominant on the property but Singapore ant was generally more prevalent in elevated positions and where bait cards were placed accordingly, Singapore ants dominated the bait cards. Very little bait was removed from either bait sample on any of the five replicates and there was no significant difference in bait remaining after 24 hours ($T=1.61$, $p>0.05$). While only small amounts of bait were actually removed, Singapore ants attended the bait cards in large numbers and appeared to be more intent on imbibing oil from the granules than actually physically removing them, as was the case for African bigheaded ant at the same site.

Greenhead Ant

Greenhead ants were present in the Brisbane cornfield where the red imported fire ant choice test was conducted. Greenhead ant dominated two of the replicates originally established for red imported fire ant. At both replicates, all bait was removed, apparently faster for Distance® Plus (mean of 68 minutes) than for Distance® (mean of 95 minutes) but the low replication prevented a meaningful statistical result for time to total removal ($T=11.1$, $p>0.05$).

Yellow Crazy Ant (*Anoplolepis gracilipes*)

Nine separate trials (4 no-choice tests, 4 choice tests and one semi-field trial) were conducted in Australia and neighbouring islands (Table 1). On Tokulau, in both the choice and no-choice trials, ants completely removed the 1 g samples of Distance® Plus within ca. 20 minutes but removed very little Distance®. As such, no statistical analysis was possible. This was also the case for the two no-choices trials on Christmas Island and the four trials at Nhulunbuy. On Christmas Island, all Distance® was removed within 2 hours but on average 92% and 98% of the Distance® samples remained. At Nhulunbuy, ants removed the 2 g samples of Distance® Plus in ca. 90

minutes in both the choice and no choice trials conducted in October 2004 and Distance® still remained on every bait card. The mean removal time for the 0.5 g samples offered in the choice trial in June 2005 was 84 minutes whereas Distance® remained on all bait cards. For the small field trial at Nhulunbuy, all Distance® Plus bait granules were removed from the two 1 m^2 plots in an average of 45 minutes while Distance® granules were still visible in the two plots at the end of the trial (1 hr). The choice test at Caboolture in April 2005 was the only trial where any Distance® Plus bait remained at the end of the trial. At the five bait cards between 75 and 100% of Distance® remained whereas Distance® Plus was removed completely from one bait card and between 35 and 75% of the offered bait remained on the other four bait cards.

Meat Ant

Four choice tests (two each) were conducted on the meat ants *Iridomyrmex sanguineus* (Katherine and Kununurra) and *Iridomyrmex purpureus* (Boort) (Table 1). In the choice test on *I. sanguineus* in Katherine, Distance® Plus was removed by ants more rapidly than Distance® (mean of 30 minutes vs 45 minutes respectively) ($T=9.49$, $p<0.01$). However, there was no clear pattern in the choice test in Kununurra where both baits were completely removed within the 6 hr trial period and estimates of bait removal at earlier times were similar for the two formulations. For the two choice tests on *I. purpureus* in Boort, there was no clear preference for either bait. In both trials, there was no significant difference between the two in terms of bait remaining although they approached significance ($T=2.42$, $p=0.07$ for May 2005 and $T=2.34$, $p=0.08$ for June 2005) and in both cases higher amounts of Distance® Plus were removed. In the May 2005 trial, both baits were completely removed from one replicate, Distance® Plus only from another, and both baits remained at the remaining 3 replicates. In the June 2005 trial, both baits were completely removed from two of the 5 replicates and both remained on the other three replicates. Both trials on *I. purpureus* were conducted late in the season (late autumn-early winter) and it is possible that cooler conditions reduced foraging activity and therefore bait removal.

Green Tree Ant

Six trials (2 choice and 4 no-choice) were conducted on green tree ant in a range of agricultural tree crops (Table 1). In the no-choice trial conducted in rambutans near Darwin, there was no significant difference in bait remaining after 20 hours ($T=1.74$, $p>0.05$). Almost no Distance® was removed by green tree ants and in two of the 4 Distance® Plus bait cups almost all bait was removed and virtually none from the other two. Three trials were conducted in a citrus orchard in Katherine in 2004. In all cases the amount of Distance® Plus removed was higher than Distance® but the difference was statistically significant in only one of the trials. The first choice trial conducted in October 2004 showed a significantly higher bait removal from the 0.5g samples for Distance® Plus ($T=2.99$, $p<0.05$) whilst the second choice trial in October and the no-choice trial conducted in November 2004 approached significance (both $T=2.11$, $p=0.1$). In the first of these trials, Distance ® Plus was completely removed from two of the five replicates but Distance® remained in all five weighboats. In the second trial both baits remained in all five pairs of weighboats. In the third trial, very little bait at all was removed and bait remained in all 5 pairs of weighboats. Two no-choice trials conducted in a mango plantation in Kununurra in August and October 2004 both showed a significantly higher bait removal for Distance® Plus. In the first trial, 10g samples were offered in plastic weighboats and removal rates estimated at 8hrs, 26hrs and 5 days after bait placement. For Distance®, bait remaining decreased to a mean of 78% over 5 days while no Distance® Plus remained at the end of this period. These differences were significant at each time period (Table 1). In the second trial, significantly more Distance® Plus was removed by green tree ants than Distance® ($T=2.8$, $p=0.04$) and Distance® Plus was completely removed from one weighboat while Distance® remained in all five weighboats.

There was evidence in at least one trial (rambutan trial in Darwin) of green tree ants actually discarding Distance® granules, once retrieved. Some individuals were observed removing granules from the bait dispenser, moving them a short distance along the branch and then dropping them to the ground. This was not observed for Distance® Plus and in fact ants were observed actively transporting granules of Distance® Plus further up into the canopy of the mango trees, presumably to the nest.

Black Ant

The data on black ants was limited. In two choice tests (Darwin and Sydney) there were no significant differences in attractiveness of the two products (Table 1). In dragonfruit, *Iridomyrmex* sp. A (*gracilis* gp) was the dominant species active on dragonfruit flowers and on bait cards with only ca. 25% of both baits removed within the 5 hour period of the trial.

Pale Tyrant Ant
On a domestic property in Cairns, ants present in the front lawn of the property were offered both baits in a choice test. All Distance® Plus samples were completely removed whereas the bulk of Distance® remained at the end of the trial (Table 1). Distance® Plus was removed rapidly (mean of 48 minutes) whereas, at no replicate was Distance® completely removed.

DISCUSSION

Most of the available corn-based formulations available in the USA, and now elsewhere in the world, were designed specifically for red imported fire ant based on the early work of Lofgren and colleagues (Lofgren *et al.* 1961, 1964, Jouvenez *et al.* 1974) which showed that corn-based granular baits using refined soybean oil as the attractant were attractive to this species. These baits are also known to be attractive to African bigheaded ant, *Monomorium* spp. and little fire ant (Vail and Williams 1995, Vail *et al.* 1996, Taniguchi *et al.* 2003, Causton *et al.* 2005, Sousa *et al.* 2008) but less attractive to some other species that have a higher affinity with protein and carbohydrate (Klotz *et al.* 2000, Rey and Espadaler 2004, Stanley 2004, Stanley and Robinson 2007).

In the trials reported here there were large differences in attractiveness of Distance® and Distance® Plus between species. For those trials where a statistically significant difference was evident, the enhanced formulation was always more attractive than the basic formulation and in all other trials, with the exception of one (black ants in Darwin), there was a trend towards this same outcome. For some species such as yellow crazy ant, green tree ant and pale tyrant ant this difference was dramatic. For other species such as red imported fire ants, tropical fire ants, Singapore ant and meat ants the difference was more incremental in nature, improving the speed of bait retrieval. Yellow crazy ant is an important invasive ant worldwide (Lowe *et al* 2001, Wetterer 2005, Hoffmann and Saul 2010, Hoffmann *et al.* 2011) and in Australia there are current eradication programs in place on Christmas Island, in Arnhem Land and around Cairns. Current programs utilise an unregistered protein granule containing either fipronil or *s*-methoprene approved under special permit in Australia (Boland et al. 2011, Hoffmann *et al.* 2010).
The nine trials included here clearly demonstrate that Distance® is not very attractive to yellow crazy ant but in all but one trial Distance® Plus was completely removed from bait stations and much more rapidly than Distance®. This indicates that the inclusion of other ingredients strongly enhances bait attractiveness to yellow crazy ant. While Distance® Plus has been trialled in these eradication programs the only published data on efficacy so far is Webb and Hoffmann (2013) where the use of Distance® Plus promoted a significantly decline in the abundance of yellow crazy ant and it would appear that yellow crazy ant has now been eradicated from that site using multiple applications of Distance® Plus (Hoffmann pers. com.).

The remaining 5 species or species-groups (green tree ant, meat ants, greenhead ant, black ants and pale tyrant ant) are native to Australia and to varying extents constitute a nuisance to agriculture or human lifestyle (Gerozisis et al. 2008). Green tree ants are most often considered to be beneficial in tree crops, attacking other pest species (Van Mele 2008, Peng and Christian 2008, Peng *et al.* 2011). However, they are also considered a nuisance to field workers during harvest periods as well as forming mutualistic relationships with honeydew-secreting insects (Van Mele 2008, Way and Khoo 1992). Hence there may be situations where control of green tree ant is desirable. The trials included here indicate that Distance® Plus was more attractive than Distance®. Even though these differences were not significant in all trials, more Distance® Plus was removed than Distance® in every trial. Similarly, meat ants and various species of black ants (*Iridomyrmex* spp.) can also be a nuisance during harvest periods in tree crops but are also well known for tending honeydew insects such as mealybugs, scale and leafhoppers and promoting sooty mold damage on crops (James *et al.* 1996, Stevens *et al.* 2002, Dao *et al.* 2014, Webb *et al.* 2013). In three of the four trials on meats ants there was a tendency towards higher removal of Distance® Plus and in the fourth trial all bait of both types was removed over a 6 hour period. In Dragonfruit in Darwin, black ants (*Iridomyrmex* sp. A (*gracilis* gp) were observed damaging the flowers and presumably impacting fruit development. This has also been observed on other crops, for example blueberries in northern NSW, where black ants physically damage the delicate petals (Webb, unpublished data). Hence, black ants are not only instrumental in the protection of honeydew-secreting insects but may also directly impact fruit production.

Some species included here are more of a nuisance in urban rather than agricultural environments (Gerozisis *et al.* 2008). Greenhead ants are common nuisance ants around suburban gardens and have a potent sting sometimes leading to allergic reactions and anaphallaxis (Solley 1990, Meyr and Brown

2010, Brown et al. 2011). Although the data here on greenhead ant is very limited, it does suggest that there may be some advantage in Distance® Plus over Distance® as the speed of removal was greater for the former. Pale tyrant ant is common across northern Australia (Hetterick and Shattuck 2011) but to my knowledge this species has not so far been noted as a pest species. However, in both Darwin and Cairns, pale tyrant ant can be a nuisance in domestic and recreational turf, creating unsightly soil mounds and uneven turf thatch (Webb unpubl. data). Distance® Plus was clearly more attractive than Distance® to pale tyrant ant in a domestic lawn in Cairns.

For some species, there was evidence that the response to the presence of ant bait may be more complex than simple bait retrieval. In Katherine, both African bigheaded ant and Singapore ant were observed aggregating on bait samples with limited removal of granules. Presumably due to worker size, granule removal was achieved largely through co-operation between individuals. At times up to 4 individuals were observed co-operatively removing granules and this is known in other species (Bennett *et al.* 2013). However, the dominant behaviour was mass aggregation on the granule samples. This suggests that they may have been either imbibing oil directly from the granules or excising small fragments before removing them. Granule size is important and some smaller species are known to selectively remove smaller particles or excise portions of granules (Hooper-Bui *et al.* 2002) or simply extract the oil from granules (Souza *et al*. 2008, Bennett *et al.* 2013). As Pyriproxyfen is oil soluble (Sullivan and Goh 2008), both products are formulated by dissolving the active ingredient in soybean oil which is then absorbed onto the corn granules in the mixing process. Therefore the effect on ant colonies may not necessarily rely on the physical collection of whole granules.

The collection of bait granules by ants also does not necessarily guarantee processing and consumption in the colony. Food collection and storage by ants is heavily dependent on colony requirements at any point in time (Rust *et al.* 2000, Cook *et al.* 2010) and so granules collected by foraging workers may be cached in the nest for later use or may in fact be rejected by the colony. Further, bait acceptance may vary according to the quality of other resources available at any point in time (Sola *et al.* 2013). The reason why green tree ant discarded Distance® granules in the rambutan trial is not clear and without further study I can only speculate that this was a response to a lower value food item, relative to Distance® Plus. It does, however, serve as a caution to the assumption that bait removal necessarily results in both bait accumulation in the nest and bait consumption.

Distance® Plus was shown to be more attractive than Distance® in a number of species including those not normally considered to be attracted to standard corn and oil-based granular baits. The improved attractancy offers the opportunity for use of Distance® Plus in eradication programs for invasive ants as well as in control programs for nuisance ant species in urban and agricultural environments.

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Table 1: Broad trial parameters and results. Some data did not conform to a normal distribution even after transformation, because all or most of the Distance Plus bait was removed and all or most of the Distance bait remained – these trials are indicated with *. For trials where time to total removal was recorded and bait remained at the end of the trial, a mean of all values is given and expressed as > than that mean. No statistical analysis was conducted on these trials and are indicated with N/A.

Location	Habitat	Time	Test Design & Replication	Bait Placement	Assessment	Distance	Distance Plus	Statistics
Red Imported Fire Ant								
Brisbane (Qld)	Corn field	April 2005	Choice Test (n=6)	1g samples on plastic cards	Time to total removal	> 283 ± 11.1 min	> 263 ± 14.9 min	N/A
					% bait remaining (5hr)	71 ± 10.3%	30 ± 17.0%	T = 3.56, df 5, p = 0.02
Tropical Fire Ant								
Darwin (NT)	Melon farm	September 2007	No Choice Test (n=5)	0.5g samples in plastic cups	Time to total removal	> 90.2 ± 12.0 min	52.8 ± 8.4 min	N/A
					% bait remaining (2hr)	16 ± 7.5%	Nil	*
Darwin (NT)	Melon farm	September 2007	No Choice Test (n=5)	0.5g samples on plastic cards	Time to total removal	> 80.2 ± 6.8 min	48.6 ± 7.1 min	N/A
					% bait remaining (2hr)	28 ± 17.4%	Nil	*
Darwin (NT)	Melon farm	June 2009	No Choice Test (n=10)	0.5g samples on plastic cards	Time to total removal	> 306 ± 24.1 min	323 ± 20.2 min	N/A
					actual bait remaining (6hr)	0.31 ± 0.08 g	0.33 ± 0.05 g	*
African Bigheaded Ant								
Katherine (NT)	Suburban blocks	October 2004	Choice Test (n=4)	0.5g samples on plastic cards	Actual bait remaining (24hr)	0.34 ± 0.09 g	0.30 ± 0.10 g	T = 1.6, df 3, P =0.21
Darwin (NT)	Jackfruit orchard	November 2004	Choice Test (n=4)	1.0g samples on plastic cards	Time to total removal	109 ± 11.2 min	76 ± 3.8 min	T = 3.80, df 3, P = 0.03
Darwin (NT)	Suburban properties	June 2005	Choice Test (n=5)	1.0g samples on plastic cards	Time to total removal	> 6 hr	> 272 ± 77 min	N/A
					Actual bait remaining (6hr)	0.66 ± 0.05 g	0.12 ± 0.09 g	T = 5.09, df 4, p < 0.01
Lord Howe Island (NSW)	Garbage tip	March 2006	No Choice Test (n=5)	0.5g directly on the ground	Time to total removal	> 109 ± 9.0 min	> 90.8 ± 12.3 min	N/A
					% bait remaining (2 hr)	45 ± 20.0%	30 ± 18.8%	T = 0.83, df 3, p=0.44
Singapore Ant								
Katherine (NT)	Suburban properties	October 2004	Choice Test (n=5)	0.5g samples on plastic cards	actual bait remaining (24hr)	0.49 ± 0.02 g	0.40 ± 0.05 g	T = 1.61, df 4, p = 0.18
Greenhead Ant								
Brisbane (Qld)	Corn field	April 2005	Choice Test (n=2)	0.5g samples on plastic cards	Time to total removal	95 ± 5.0 min	67.5 ± 12.5 min	T = 11.1, df 1, p = 0.06
					% bait remaining (5hr)	Nil	Nil	N/A
Yellow Crazy Ant								
Nukunonu Atoll, Tokulau	Village surrounds	September 2004	Choice Test (n=5)	1.0g samples on plastic cards	Time to total removal	>45min	19.8 ± 1.7 min	N/A
					% bait remaining (45min)	91.3 ± 5.5%	Nil	*
			No Choice Test (n=5)	1.0g samples on plastic cards	Time to total removal	>45min	22.6 ± 4.4 min	N/A
					% bait remaining (45min)	94.6 ± 2.2%	Nil	*
Christmas Island	Natural rainforest	September 2004	No Choice Test (n = 5)	1g sample on plastic cards	actual bait remaining (2hrs)	0.92 ± 0.03 g	Nil	*
Christmas Island	Natural rainforest	September 2004	No Choice Test (n = 5)	1g sample on plastic cards	actual bait remaining (2hrs)	0.98 ± 0.02 g	Nil	*
Rocky Bay, Nhulunbuy (NT)	Natural rainforest	October 2004	Choice Test (n=4)	2g samples on plastic cards	Time to total removal	> 24 hrs	87 ± 14.7 min	N/A
					% bait remaining (24 hrs)	78 ± 8.5%	Nil	*
Rocky Bay, Nhulunbuy (NT)	Natural rainforest	October 2004	No Choice Test (n=4)	2g samples on plastic cards	Time to total removal	> 3 hr	88 ± 20.8 min	N/A
					actual bait remaining (3hr)	2.1 ± 0.05 g	Nil	*
Rocky Bay, Nhulunbuy (NT)	Natural rainforest	October 2004	Field Test (n=2)	Bait spread at 10kg/ha over 1m^2 plots	Time to total removal	> 60 min	45 ± 5 min	N/A
					% bait remaining (1 hr)	100%	Nil	N/A
Rocky Bay, Nhulunbuy (NT)	Natural rainforest	June 2005	Choice Test (n=5)	0.5g samples on plastic cards	Time to total removal	> 98.6 ± 1.9 min	83.6 ± 3.1 min	N/A
					% bait remaining (4hrs)	0.27 ± 0.05g	Nil	*
Caboolture (Qld)	Industrial complex	April 2005	Choice Test (n=5)	1g samples on plastic cards	Time to total removal	> 249 ± 3.3 min	> 201 ± 32.1 min	N/A
					% bait remaining (4 hr)	95 ± 5%	35 ± 15%	*
Meat Ant								
Katherine (NT)	Citrus orchard	October 2004	Choice Test (n=5)	1g samples on plastic cards	Time to total removal	45 ± 5.4 min	30 ± 6.5 min	T = 9.49, df 4, p < 0.01
Boort (Vic)	Olive grove	May 2005	Choice Test (n=5)	1g samples on plastic cards	Time to total removal	>291 ± 9.0 min	>249 ±32.9 min	N/A
					% bait remaining (5hr)	54 ± 18.6%	30 ± 16.7%	T = 2.42, df 4, p = 0.07
Boort (Vic)	Olive grove	June 2005	Choice Test (n=5)	1g samples on plastic cards	Time to total removal	> 255 ± 29.4 min	> 243 ± 38.1 min	N/A
					actual bait remaining (4hr)	0.41 ± 0.18 g	0.20 ± 0.12 g	T = 2.34, df 4, p =0.08
Kununurra (WA)	Mango plantation	July 2004	Choice Test (n=6)	2g samples in plastic weighboats	% bait remaining (6hr)	0%	0%	N/A

Green Tree Ant								
Darwin (NT)	Rambutan orchard	November 2004	No Choice Test (n=4)	1g samples in plastic cups	actual bait remaining (20hr)	0.95 ± 0.02g	0.58 ± 0.23 g	T = 1.74, df 3, p = 0.18
Katherine (NT)	Citrus orchard	October 2004	Choice Test (n=5)	0.5g samples in plastic weighboats	actual bait remaining (17hr)	0.49 ± 0.01 g	0.22 ± 0.09 g	T = 2.99, df 4, p = 0.04
Katherine (NT)	Citrus orchard	October 2004	Choice Test (n=5)	0.5g samples in plastic weighboats	actual bait remaining (24hr)	0.45 ± 0.03 g	0.33 ± 0.05 g	T = 2.11, df 4, p = 0.1
Katherine (NT)	Citrus orchard	November 2004	No Choice Test (n=5)	1g samples in plastic weighboats	actual bait remaining (19hr)	1.04 + 0.02 g	0.86 ± 0.08 g	T = 2.11, df 4, p = 0.1
Kununurra (WA)	Mango orchard	August 2004	No Choice Test (n=5)	10g samples in plastic weighboats	% bait remaining (8hrs)	93 ± 4.3 %	50 + 7.6 %	T = 4.92, df 4, p < 0.01
					% bait remaining (26hrs)	84 ± 7.5 %	8 ± 2.5 %	T = 9.61, df 4, p < 0.001
					% bait remaining (5 days)	78 ± 8.6 %	0 %	T = 9.07, df 4, p < 0.001
Kununurra (WA)	Mango orchard	October 2004	No Choice Test (n=5)	0.5g samples in plastic weighboats	actual bait remaining (24hr)	0.57 ± 0.01 g	0.29 ± 0.10 g	T = 2.8, df 4, p = 0.04
Black Ant								
Cronulla (NSW)	Suburban property	September 2004	Choice Test (n=3)	1g samples in plastic weighboats	% bait remaining (44hr)	0.5 ± 0.06 g	0.23 ± 0.12 g	*
Darwin (NT)	Dragonfruit orchard	November 2004	Choice Test (n=5)	1g samples on plastic cards	% bait remaining (5hr)	73 ± 0.09 %	75 ± 0.09%	T = -1.0, df 3, p = 0.39
Pale Tyrant Ant								
Cairns (Qld)	Suburban property	November 2004	Choice Test (n=5)	1g samples in plastic weighboats	Time to total removal	> 150 min	48 ± 14.4 min	N/A
					actual bait remaining (3hr)	0.93 ± 0.08 g	Nil	*