Field Evaluations of the Efficacy of Distance Plus on Invasive Ant Species in Northern Australia

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J. Econ. Entomol. 106(4): 1545-1552 (2013); DOI: http://dx.doi.org/10.1603/EC13094 ABSTRACT The efficacy of Distance Plus Ant Bait, containing the insect growth regulator pyriproxyfen, was tested in the field against two invasive ant species in northern Australia: African big-headed ant (*Pheidole megacephala* (F.)) and yellow crazy ant (*Anoplolepis gracilipes* (Fr. Smith)). Results were also gained for a third pest species, Singapore ant (Monomorium destructor (Jerdon)), from one trial focused primarily on P. megacephala. Five studies were conducted throughout northern Australia, each with different protocols, but common to all was the broad-scale dispersal of Distance Plus, coupled with long-term monitoring of ant population levels. Additionally, a laboratory trial was conducted to assess if there was a direct toxic effect by the bait on A. gracilipes workers, and ant community data were collected at some sites in the A. gracilipes trial to assess nontarget impacts and subsequent ecological recovery. All three species were greatly affected by the treatments. The abundance of *P. megacephala* declined dramatically in all trials, and by the final assessment for each study, very few ants remained, with those remaining being attributable to edge effects from neighboring untreated properties. At both sites that it occurred, M. destructor was initially at least codominant with P. megacephala, but by the final assessment, only three M. destructor individuals were present at one lure at one site, and only a single individual at the other site. Abundance of A. gracilipes fell, on average, to 31% of control levels by 91 d and then slowly recovered, with subsequent treatments only providing slightly greater control. No direct toxic effect on workers was found in the laboratory trial, indicating that population declines of A. gracilipes were typical bait-related declines resulting from reduced worker replacement. Nontarget impacts of the bait could not be distinguished from the negative competitive impacts of A. gracilipes, but there was a noticeable absence of some key common ant species posttreatment, which was more likely the result of baiting rather than competitive exclusion. The species composition of treated and untreated sites was statistically indistinguishable in multivariate analysis within 2 yr posttreatment, indicating ecological recovery. Our findings indicate that Distance Plus has great potential for invasive ant management.

KEY WORDS ants, control, eradication, invasive ants, treatment

Many ant species have become widely dispersed and problematic throughout the world (McGlynn 1999, Holway et al. 2002), increasingly requiring management action for control or eradication (Hoffmann et al. 2010, 2011). A range of consumer and professional pest control products, largely based on toxic active constituents such as boron, fipronil, and hydramethylnon, are available, but broad-scale management actions are increasingly requiring products that are more environmentally "friendly," with less potential of nontarget impacts and especially implications for human health.

Insect growth regulators (IGRs), specifically those that act as juvenile hormone mimics (e.g., methoprene, fenoxycarb, and pyriproxyfen), interfere with the reproductive biology and colony dynamics of ants (Klotz et al. 2008). They have been widely used for control of red imported fire ant (*Solenopsis invicta* Buren) in the United States, Australia, and Taiwan (Williams et al. 2001, Vanderwoude et al. 2003, Hwang 2009), and they are also known to be effective on other invasive species such as African bigheaded ant (*Pheidole megacephala* (F.)), pharaohs ant (*Monomorium pharaonis*), and little fire ant (*Wasmannia auropunctata* (Roger)) (Edwards 1975, Horwood 1988, Reimer and Beardsley 1990, Vail and Williams 1995, Vail et al. 1996, Hsieh and Su 2000, Lee 2002, Lee et al. 2003, Lim and Lee 2005, Souza et al. 2008).

Pyriproxyfen is known to be a potent inhibitor of reproduction and fecundity in *P. megacephala, M. pharaonis*, and *S. invicta* in the laboratory (Glancey et al. 1990, Banks and Lofgren 1991, Reimer et al. 1991, Vail and Williams 1995), and it is therefore likely to be effective in the field. However, to our knowledge, there are few published reports of field efficacy of pyriproxyfen on invasive or nuisance ant species, other than *S. invicta*. Souza et al. (2008) achieved field suppression of *W. auropunctata* in an orchard envi-

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ronment using Distance Ant Bait, the original nonenhanced U.S. formulation. Similarly, Vail et al. (1996) succeeded in reducing foraging *M. pharaonis* by >85% in an urban environment using a perimeter treatment of corn and oil-based pyriproxyfen granules. There have also been other studies where pyriproxyfenbased baits have been used in conjunction with other treatments for successful eradication or population suppression, but the individual effects of pyriproxyfen were not recorded (Taniguchi et al. 2003, Hoffmann et al. 2011).

Given the clear evidence from laboratory and field trials of the efficacy of pyriproxyfen-based baits on *P. megacephala* and other species elsewhere, we anticipate similar efficacy against invasive or nuisance ants in Australia. Here we report the results of five independent studies conducted throughout tropical northern Australia to evaluate the efficacy of Distance Plus on three key invasive ant species in urban, agricultural, and natural environments: *P. megacephala*, Singapore ant (*Monomorium destructor*), and yellow crazy ant (*Anoplolepis gracilipes*).

Materials and Methods

Each study was conducted with different protocols, detailed in the following text, but consistent among them was: 1) all work was conducted between 6:30 and 9:00 a.m. or after 4:00 p.m., when temperatures are cool enough to not adversely affect ant activity; 2) ant abundance at lures was scored according to the following scale: 0 = no ants, 1 = 1 ant, 2 = 2-5 ants, 3 =6-10 ants, 4 = 11-20 ants, 5 = 21-50 ants, 6 = 50-100ants. 7 = >100 ants to aid the rapid counting of ants at lures in the field; 3) all assessments were conducted at 15 min after lure placement, and abundance data were averaged across the lures to give a single abundance value for each site at each sample time. Lures varied according to species. A small scoop of tinned tuna $(\approx 2 \text{ g})$ was used for A. gracilizes and the P. megacephala trial in Nhulunbuy, whereas a small scoop of peanut butter (≈ 2 g) was used for *P. megacephala* (and *M. destructor*) in the remaining three trials.

Pheidole megacephala. The response of P. megacephala to Distance Plus was quantified in four independent studies in the Northern Territory. The first study used four spatially independent suburban properties, each 800-1,000 m², contained within larger infested areas of the township of Nhulunbuy. Three of these properties were broadcast treated using a handheld applicator on 10 December 2004 at the rate of 4 kg/ha. The remaining property was left untreated as a control. The weather on the day of bait distribution was warm (~33°C), humid, and overcast, and without rain for at least 24 h after bait application. Activity of P. megacephala was quantified on each property at 10 randomly placed lures 15 min after placement on the day before treatment and at 14, 60, and 101 d posttreatment.

The second study used four spatially independent suburban properties in Darwin ($\approx 800 \text{ m}^2$) together with a 1 ha block on the nearby government agricul-

tural research facility. Three of suburban blocks and the 1 hablock were treated with Distance Plus, and the remaining suburban block was used as a control. Bait was broadcast by hand over the entire area of the three properties and the 1 ha block at the rate of 4 kg/ha on the morning of 24 November 2004. The weather on the day of bait distribution was warm (\approx 34°C), humid, and overcast, and no rain occurred for at least 24 h after bait application. Activity of *P. megacephala* was quantified on each property at five lures after 15 min, on the treatment day, 28 and either 84 or 93 d posttreatment, except for the control site, which was not sampled on the second assessment, and sampled at 96 d posttreatment.

The third study treated five spatially independent suburban residences in Katherine, with bait broadcast by hand at a rate of 4 kg/ha on the morning of 24 February 2005, except one site, which was treated on 23 March 2005. No control population was sampled in this study. The weather conditions and sampling methodology were the same as the second study, but the posttreatment samples occurred after 27 and 56 d, with those of the site with the different treatment date offset accordingly. Activity was quantified on lures 15 min after placement. Two sites in this trial also contained high numbers of *M. destructor*, such that it was dominant at one site and codominant at the other. Individual counts were not made for these two species, so we consider their responses to the treatment together.

The fourth study treated a small jackfruit plantation (6 rows of 10 trees, $\approx 2,000 \text{ m}^2$) in Darwin's rural area with bait hand-broadcast at a rate of 4 kg/ha on the morning of 2 November 2004. The weather conditions and sampling methodology were the same as the second study, but sampling occurred after 8, 42, and 98 d posttreatment. No control population was sampled in this study, but the trial was conducted concurrently with nearby trial 2. Activity was quantified on lures 15 min after placement.

Anoplolepis gracilipes. Field Trials. Treatments for A. gracilipes were conducted on the entirety of six spatially discrete infestations around Nhulunbuy (Northern Territory) ranging in size from ≈ 0.1 to 23 ha from May 2009 to September 2010. All treatments were conducted aerially by helicopter using an underslung dispersal bucket, and also treated a 100-m buffer zone around each site, with the exception of an island that was treated to the shore line. Sites were divided into three treatment regimens, a single treatment at one site, a double treatment at one site, and triple treatment at four sites (Fig. 1), with treatments applied at the rate of 5 kg/ha. Where multiple treatments were applied, the intention was to space treatments apart by 3 mo, but this was not always possible. The triple treatment regimen was timed so that the first treatment occurred after A. gracilipes sexual brood had completed development. In the first year (2009), the triple treatment trial was abandoned after it appeared that the second treatment had no effect, possibly because it was conducted too soon after the first treatment. Because the A. gracilipes populations had fully recovered (almost all lures having maximum ant



Fig. 1. Treatment and sampling regimen for the field trial and ecological assessments for *Anoplolepis gracilipes* in northeast Arnhem Land. Letters in boxes indicate the site code (A–C) of sites used for ecological assessments, and the arrows indicate the treatment months. Broken lines indicate sampling times of the nontarget impact surveys. B* indicates that this treatment at site B used a bait containing fipronil, not Distance Plus. Three other sites were treated, the first being a single treatment site that was treated only in March 2009, and the remaining two were triple-treated sites, treated at identical times as site C.

scores) in these sites by the beginning of the second year (2010), the same sites were used again, with the first 2010 treatment being considered to be the first treatment. Concurrently, throughout the 2 yr, an additional five untreated sites, also independent infestations, were monitored as controls.

Abundance of A. gracilipes was quantified at each site at 11 lures, spaced 10 m apart along a 100-m transect. In some instances, this design was not possible, and the distance between lures and the shape of the transect was modified, the most extreme being at the smallest site, which had two parallel transects with baits spaced ≈ 3 m apart. Assessments were conducted at irregular intervals before and throughout the treatment regimens, with a general frequency of weekly after the first treatment to tri-monthly toward the study's cessation. All sites were monitored for approximately 9 mo after the final treatment.

Laboratory Trial. Because the IGR treatments made A. gracilipes abundance decline more rapidly than expected in the field trials, we tested for direct toxic effects in the laboratory. Ten clear plastic containers (8 cm diameter, 10 cm high), with a moist piece of kitchen sponge (2 cm by 2 cm) wired to the inside of the lid to maintain humidity, were used to each house ≈ 150 workers. A box was then placed over all of the containers to create a dark environment, simulating a nest situation. The ants were first allowed to settle into the trial containers for 24 h, and then five granules of Distance Plus were added to five of the containers. Nothing was added to the other five control containers. The number of dead ants in each container was counted at the end of each 24 h period for 4 d. Because the number of ants in each container was not the same, effects were compared by comparing the percentage of dead workers between the two treatments after 4 d using the nonparametric Mann-Whitney U test.

Nontarget Impacts. Ecological data were obtained from three of the treatment sites and at the five untreated control sites without *A. gracilipes* to quantify nontarget impacts and subsequent ecological recovery. The three treatment sites were treated thrice with Distance Plus (site A), twice with Distance Plus followed by a treatment using a product that contained fipronil (site B), or five times with Distance Plus (site C) (Fig. 1). For the controls, in 2010 and 2011, only two sites were sampled, and these were within a slightly different habitat to the treatment sites. For greater comparison, three additional control sites were sampled in 2012, positioned nearby to and within the same habitat as the three treatment sites. Ants were sampled at each site using a 5 by 3 grid of pitfall traps (4.5 cm diameter, partly filled with ethylene glycol as a preservative) with 10 m spacing, operated for 48 h. No pretreatment data were obtained, with sample dates being October 2010, August 2011, and September 2012 (Fig. 1). Ants were sorted and counted in the laboratory at species level, and species names and codes used here follow those used in the Commonwealth Scientific and Industrial Research Organization Ecosystem Sciences ant collection in Darwin.

Analysis. For the A. gracilipes trial, because some treatments were repeated in a second year using a different annual timing, replicate treatments could not be directly compared or combined owing to the confounding influence of seasonality on A. gracilipes abundance. Likewise, the nonorthogonal study design and low replication precluded the analysis of treatment types as categorical variables. Instead, all replicates were combined into a single analysis using time since treatment as a continuous variable, with A. gra*cilipes* abundance within each replicate expressed as the relative proportion of the controls for each sample time. This approach allowed the inclusion of data from sites having multiple treatments to be first included in the categories of fewer treatments, thereby improving the replication. Unfortunately, statistical analysis using the nonparametric Mann–Whitney U test at individual times only had enough replicates to be appropriately meaningful for the first sample. The limitation is that the data are not specific for any particular time of year or for any part of the A. gracilipes reproductive cycle, and they therefore only illuminate broad patterns.

In the laboratory trial, percentage worker mortality after 4 d was compared between the treatment and control groups using a Mann–Whitney *U* test. For the ecological data assessing nontarget impacts, because of the low site-level replication, statistical analysis was restricted to comparing the species composition of treated and untreated sites using ANOSIM (Analysis of Similarity) within an nMDS (nonmetric multidimensional scaling) based on a Bray–Curtis association matrix of presence or absence data.

The results of all *P. megacephala* trials were combined into a single time since treatment graph. Owing to the lack of controls in some trials, and the use of only a single control site when present, no statistical analyses were performed.



Fig. 2. Average scaled *Pheidole megacephala* abundance at treatment (closed symbols) and control (open symbols) sites in the four trials (trial 1: square; trial 2: diamond; trial 3: circle; trial 4: triangle).

Results

P. megacephala. P. megacephala abundance displayed a clear decline after treatment up to the longest sampling time of 101 d, greatly contrasting with the consistently high abundance in the controls, which were almost always the highest scores (Fig. 2). By the final assessment for each study, few ants remained present, with those present often being attributable to edge effects from neighboring untreated properties. In trial 1, P. megacephala was present at only 3 of 30 lures, having only 1, 2, and 0 ants, respectively, in sites 1, 2, and 3. In trial 2, there was a mean count of two ants per lure in the final assessment, compared with 107 ants per lure at trial commencement, and two of the four treated sites had no ants in the final assessment. In the final assessment of trial 3, P. megacephala was present at only 6 of 40 lures, predominantly at low levels (<5 ants). In trial 4, by 98 d posttreatment, there were only two *P. megacephala* individuals at a single lure, and no other ant species were observed in the plantation.

M. destructor. M. destructor was clearly affected at the two sites in trial 3 that it infested. Although the abundance of *M. destructor* was not quantified separately from *P. megacephala*, at both sites, *M. destructor* was initially codominant with *P. megacephala*. After treatment, the abundance of all ants was reduced by >99%. By the final assessment, only three *M. destructor* individuals were present at one lure in the first site, and only a single individual in the second site.

A. gracilipes. Field Trials. Before treatments, A. gracilipes abundance in treatment and control sites was statistically indistinguishable (Mann–Whitney U test; U = 9.5; z = -0.52; P = 0.6), but after a single treatment, abundance within treatment sites fell, on average, to 31% of control levels by 91 d (Fig. 3). When only a single treatment was applied, the ant popula-

tions appeared to stabilize after ≈ 50 d. Second and third treatments, on average, did not provide a greater effect, but there is a pattern that sites treated thrice took longer to recover than sites treated twice, with abundance remaining at approximately one-third of control levels after almost 1 yr. Eradication was not achieved at any site.

Laboratory Trial. Worker mortality in the containers after 4 d varied from 7.7 to 22.9%. There was no difference between the mortality rates of the two treatments (Mann-Whitney U-test: U = 7, Z = -1.04, P = 0.3), indicating that the ant bait had no immediate toxic effect, and therefore population decline seen in the field trials could be attributable to typical IGR effects such as reduced fecundity and reduced worker replacement.

Nontarget Impacts. Ordination of species presence or absence data showed that immediately after the treatment regimen in 2010, the treated sites are widely dispersed away from the control sites (Fig. 4). This separation reflects the absence of a few highly common ant species, particularly Iridomyrmex reburrus Shattuck, Iridomyrmex pallidus Forel, Melophorus sp. one *aeneovirens* species-group, and *Monomorium* sp. 24 laeve species-group. Site C, which had undergone five treatments over 2 yr, was most distant from the controls. Notably, species richness in treated sites immediately after treatments in 2010 ranged from 9 at site C to 20 at site B, which was identical or greater than species richness levels in 2012 for both treated and control sites. In subsequent years, all treated sites shifted toward the three more comparable controls (C3-C5) sampled in 2012. In 2012, the species richness within treatment sites ranged from 9 to 15, and in the controls from 6 to 13 (sites C3-C5), and the community composition of the three treatment sites was statistically indistinct from all of the controls (ANOSIM:



Fig. 3. Mean (\pm SE) percent *Anoplolepis gracilipes* abundance at treatment sites relative to abundance at control site, at sites treated once (circles) twice (triangles), or thrice (squares) with Distance Plus.

Global R = -0.2, P = 0.857), indicating ecological recovery had occurred.

Discussion

These studies clearly demonstrated that Distance Plus had a dramatic effect on the abundance of all three species. Baiting had a significant impact on *P. megacephala* in all four trials, and although somewhat anecdotal, *M. destructor* succumbed to baiting in the two sites in which it occurred. Results against *A. gracilipes* were not as dramatic as for the other two species, but this species was clearly affected at least in the short- to medium-term (1–3 mo). It was envisaged that a triple treatment might have been sufficient to achieve eradication of entire infestations, but this was not so, even after five treatments over 2 yr. Nevertheless, a small-scale eradication of this species covering ≈ 0.25 ha within a residential area within the city of Darwin was recently achieved in 2009 using only Distance Plus (A. Walters, personal communication), demonstrating that it is possible. Interestingly, the most substantial reduction in *A. gracilipes* populations occurred after the first treatment, which raises ques-



Fig. 4. NMDS ordination of sites based on ant species presence or absence data at treated sites (gray circles) treated with Distance Plus thrice (site A), twice and then with a bait containing fipronil (site B), five times (site C), and untreated controls (C1–C5; black circles). Numbers on the site codes indicate the sample year (2010–2012). 2D stress = 0.15.

tions about the biological response of *A. gracilipes* to baiting, particularly multiple applications, especially how it differs relative to *P. megacephala* and *M. destructor*. Possible mechanistic causes include bait shyness, application timing relative to social dynamics, food processing and dissemination pathways, worker to queen ratios, and nest versus queen nutrition requirements. Investigations into all such aspects of colony dynamics and how it affects bait efficacy would no doubt yield important breakthroughs for treating ants.

The action of pyriproxyfen on invasive ants is slow relative to compounds that induce neurotoxic effects or metabolic inhibition, as it does not directly affect foraging workers, rather it reduces the fecundity of reproductives and therefore the ability of the colony to replace workers (Klotz et al. 2008). For P. megacephala, Reimer et al. (1991) recorded reduced fecundity within 3 wk of exposure to pyriproxyfen, with 66% of queens no longer producing eggs, and by 6 wk, no eggs were produced by any queen, even those that survived for 5-6 mo after treatment. We anticipated that the reduction in the populations of the three species tested would not occur for at least a few weeks to account for brood already present, but reductions were clearly evident for A. gracilipes within days of treatments, and the same can be interpreted from the data for *P. megacephala*. In the absence of worker mortality in the laboratory experiment, we suspect that the rapid effects we found in the field trials are a result of larvae, particularly final-stage larvae, also being affected.

The apparent efficacy of Distance Plus on M. destructor found here is consistent with the known efficacy of pyriproxyfen and other juvenile hormone mimics on its congener M. pharaonis (Edwards 1975, Vail and Williams 1995, Vail et al 1996, Hsieh and Su 2000, Lee 2002, Lee et al. 2003, Lim and Lee 2005). To our knowledge, effective delivery of bait toxicants to Monomorium spp. using a corn matrix has not been demonstrated previously. Lee (2002) found that liquid matrices were preferred over gels and pastes, but high-oil-content peanut butter was also more attractive than honey to both *M. pharaonis* and *M. destructor* in southeast Asia. Therefore, it is not surprising that the corn matrix used here containing $\approx 17\%$ soybean oil was attractive to Monomorium. Indeed, bait attractancy studies (G.A.W., unpublished data) show that Monomorium spp. have a high affinity to the corn and oil matrix over alternative granules containing a high loading of both protein and sugar. They also do not necessarily transport the granules back to the nest; rather, they were commonly observed apparently imbibing oil (containing the active ingredient) from the surfaces of the granules. Hence colony effects may occur without the physical transport of the granule back to the nest.

The remarkably consistent results for *P. mega-cephala* are particularly noteworthy because of the nonconformity of methods and treatment times among the independent studies. Furthermore, all treatment areas were contained within larger infestations and therefore were subject to boundary effects.

Indeed most *P. megacephala* individuals found in the final assessment of most locations were considered to be foragers from beyond the treatment boundary. The treatment of a portion of an infestation, rather than an entire infestation, is likely to be the most common situation for ant control, especially within urban environments, and thus reinvasion will always be an issue. This raises the question of whether perimeter treatments, particularly using an IGR, might be effective as secondary barrier treatments, preventing reinfestation.

Perimeter treatments have been explored as a control option for a range of ants species (Forschler and Evans 1994a,b, Blachly and Forschler 1996, Silverman and Roulston 2003, Taniguchi et al. 2005, Aubuchon et al. 2006, Taniguchi et al. 2006, Arakaki et al. 2009). For Argentine ant (*Linepithema humile*), perimeter treatments using containerized hydramethylnon-based bait in urban environments have met with some success, but these studies have usually sought to exclude foraging workers from entering buildings. Aubuchon et al. (2006), aiming to eliminate colonies of S. invicta within 0.4-ha pasture plots using only a perimeter treatment of Extinguish (0.5% s-methoprene), had marginal success, achieving a 21% reduction in mounds and 36% reduction in ant abundance. In Hawaii, where *P. megacephala* is a pest of agricultural crops like pineapples and coffee, several studies have been conducted to evaluate the ability of perimeter bait treatments using hydramethylnon-based baits to prevent reinvasion and colony establishment after the plots had been cleared of ants. Owing to the short ultraviolet (UV) half-life of hydramethylnon (Vander Meer et al. 1982, Mallipudi et al. 1986), the focus has been on delivery through bait stations to protect the active ingredient from excessive UV exposure. Arakaki et al. (2009) successfully prevented P. megacephala from reinfesting a previously cleared coffee field using sentinel bait stations containing Amdro. However, Taniguchi (2011) was unsuccessful in maintaining a clear pineapple field when using clumped bait applications (without physical bait stations), indicating that the UV protection afforded to the bait by the bait station is important during that period of detection and recruitment to the bait. As pyriproxyfen is relatively UV stable (Sullivan and Goh 2008), Distance Plus, used as a perimeter broadcast treatment, is likely to remain effective in intercepting foraging workers. However, this concept remains to be tested.

The focus of invasive ant management is increasingly within areas of conservation value, and so it is important that any product used does not have significant nontarget effects (Hoffmann and O'Connor 2004, Hoffmann et al. 2010, Gaigher et al. 2013). Unfortunately, because of the lack of pretreatment sampling, we were unable to determine the relative contributions of *A. gracilipes* and the treatments to the differences in the native ant fauna between the treated areas and the controls soon after treatment. However, the absence of at least *Melophorus* sp. one *aeneovirens* species-group and *Monomorium* sp. 24 *laeve* species-group from the site that underwent five August 2013

treatments over 2 yr was likely the result of the baiting. This is because 1) both species occurred in the plots within 1 yr of the cessation of baiting, and 2) both are known to not be affected by *A. gracilipes* (Hoffmann and Saul 2010), presumably because of temporal separation of foraging times by *Melophorus* and the small size of *Monomorium*. Analysis of the ant community data showed that any nontarget issues occurring here were remedied within 1–2 yr, showing that any such negative effects are short-term, at least within relatively small areas of intact natural environments, as has been found previously for ant communities subject to eradication measures in northern Australia (Hoffmann 2010).

Eradication of entire populations is the ultimate outcome for pest management, and all three species assessed here are currently targets of eradication campaigns in numerous locations globally. IGR-based products offer more targeted solutions for the many situations where there is a high risk of nontarget impacts, such as on islands and around wetlands (O'Dowd et al. 2003, Lester and Tavite 2004, Hoffmann and Saul 2010) that harbor crustaceans that are particularly vulnerable to toxic ant baits. Distance Plus appears to have great potential for invasive ant management, and future work will now focus on refining the treatment protocols to better enhance an already clearly demonstrated efficacy.

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