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# Let them eat termites—prey-baiting provides effective control of Argentine ants, *Linepithema humile*, in a biodiversity hotspot

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## Abstract

Invasive ants threaten biodiversity, ecosystem services and agricultural systems. This study evaluated a prey-baiting approach for managing Argentine ants in natural habitat invaded by Argentine ants. Blackmound termites (*Amitermes hastatus*) were topically exposed to fipronil and presented to Argentine ants (*Linepithema humile*). In laboratory assays, *L. humile* colonies were offered fipronil-treated termites within experimental arenas. The termites were readily consumed, and results demonstrate that a single termite topically treated with 590 ng fipronil is capable of killing at least 500 *L. humile* workers in 4 days. Field studies were conducted in natural areas invaded by *L. humile*. Fipronil-treated termites scattered within experimental plots provided rapid control of *L. humile* and ant densities throughout the treated plots declined by 98 ± 5% within 21 days. Results demonstrate that the prey-baiting approach is highly effective against *L. humile* and may offer an effective alternative to traditional bait treatments. Furthermore, prey-baiting offers environmental benefits by delivering substantially less toxicant to the environment relative to current control methods which rely on commercial bait formulations and may offer greater target specificity.

**KEYWORDS**

*Amitermes hastatus*, Argentine ant, blackmound termite, fipronil, *Linepithema humile*, prey-baiting

## 1 | INTRODUCTION

Invasive ants are considered a significant threat to urban, agricultural and natural habitats worldwide (Holway, Lach, Suarez, Tsutsui, & Case, 2002; Lach & Hooper-Bui, 2010; Lowe, Browne, & Boudlejas, 2000). Among invasive ants, the Argentine ant, *Linepithema humile*, is one of the most problematic invaders (Silverman & Brightwell, 2008). This is mainly due to its widespread global distribution, high local abundance and high potential to cause ecological and economic damage (Holway et al., 2002; Roura-Pascual et al., 2004; Suarez, Holway, & Case, 2001). Argentine ants form fast-growing, high-density colonies which place significant pressures on native ecosystems and alter ecological processes within those ecosystems (Holway et al., 2002). The primary effect of Argentine ants is the displacement of native ant species (Rowles & O'Dowd, 2007; Sanders, Gotelli, Heller, & Gordon, 2003) and the cascading effects on other ecosystem levels (Holway et al., 2002; Walters, 2006).

Throughout their introduced range, Argentine ants are mainly associated with anthropogenic environments, disturbed habitats, and are frequently a nuisance pest in urban environments (Rust & Knight, 1990) and agriculture (Daane et al., 2008; Silverman & Brightwell, 2008). However, Argentine ants are also highly adept at invading natural environments with relatively little human disturbance (e.g., Cole, Medeiros, Loope, & Zuehlke, 1992; Holway, 1998; Ward & Harris, 2005). These ants have successfully invaded some anthropogenically influenced areas within protected areas in one of the most iconic native habitats, the Cape Floral Region in South Africa (Lach, 2013; Mothapo & Wossler, 2017). This protected area, together with its beautiful and distinctive fynbos vegetation, is one of the world's biodiversity "hotspots" (Myers, Mittermeier, Mittermeier, da

  

Fonseca, & Kent, 2000). The Cape Floral Region is currently facing a number of challenges including invasive species, fire, climate change and urban expansion (UNESCO 2009). Among invasive species, Argentine ants are a major threat to the fynbos ecosystem. They displace important seed-dispersing ant species (Bond & Slingsby, 1984; Christian, 2001) and deter pollinators from native plants that depend on insect pollination (Lach, 2008; Mothapo & Wossler, 2017). Argentine ants aggressively harass Cape honeybees from access to floral nectar in proteas (Lach, 2008), and due to their active foraging through the day and night (Human and Gordon 1996), Argentine ants often deplete the nectar in flowers before honeybees have access to it (Buys, 1987). Furthermore, the synergy between multiple stressors affecting the fynbos ecosystem is becoming increasingly evident. For example, alien tree canopy density (mainly *Pinus* and *Eucalyptus* species) and Argentine ants act synergistically to produce negative effects on the local ant fauna (Schoeman & Samways, 2013).

When Argentine ants invade sensitive environments such as national and state parks, nature reserves and wilderness areas, control options are often limited because of potential exposure of non-target organisms to insecticide residues. Non-target impacts are often a serious concern in eradication programmes in conservation areas (Hoffmann, Luque, Bellard, Holmes, & Donlan, 2016). In these situations, the use of toxic baits is the most appropriate approach for control because baits are typically contained within bait stations that minimize non-target risks (Gentz, 2009; Hoffmann et al., 2016; Silverman & Brightwell, 2008). Toxic baits are popular in controlling invasive ants and have been used to control a wide range of species (e.g., Buczkowski, Roper, & Chin, 2014; Buczkowski, Roper, Chin, Mothapo, & Wossler, 2014; Causton, Sevilla, & Porter, 2005; Daane et al., 2008; Drees, Alejandro, & Paul, 2013). Despite some successes with toxic baits (e.g., Hoffmann, 2010; Lester & Keall, 2005), baits suffer a number of disadvantages that limit their use. These include a relatively short lifespan under field conditions, susceptibility to environmental factors, ecological contamination, lack of effective dispensers and non-target effects (Silverman & Brightwell, 2008).

To improve the efficacy and safety of invasive ant management in conservation areas, more research is needed on new bait active ingredients, bait formulations and bait delivery methods. Previous research efforts have focused largely on developing new active ingredients and new bait formulations as driven by market demands for controlling nuisance ants in urban situations. Relatively little work has been done on developing new bait delivery methods, especially those suitable for large-scale, area-wide control programmes in natural areas. Recently, however, new developments have been made towards improving bait delivery methods. One example is the newly developed water-storing crystals (hydrogels) (Buczkowski, Roper, & Chin, 2014; Buczkowski, Roper, Chin, Mothapo et al., 2014). Hydrogel baits containing 0.0007% thiamethoxam were highly effective against Argentine ants in laboratory (Buczkowski, Roper, Chin, Mothapo et al., 2014) and field (Buczkowski, Roper, & Chin, 2014) trials and were successful in controlling Argentine ants in the Channel Islands, California (Boser et al., 2014). Another improvement in bait delivery methods for controlling invasive ants is the development of prey-baiting (Buczkowski, 2016, 2017). Prey-baiting takes advantage of the predatory (or omnivorous) feeding habits of many invasive ants and uses live, insecticide-treated prey to deliver the toxicant to the target species. Prey-baiting using live, fipronil-treated termites was highly effective against Asian needle ants *Brachyponera chinensis* (Emery) in laboratory and field trials (Buczkowski, 2016). A major advantage of prey-baiting is that it offers greater bait selectivity and consequently greater target specificity (Buczkowski, 2017). Additionally, prey-baiting provides environmental benefits with regard to pesticide residues in ecologically sensitive environments because it uses significantly less toxicant relative to traditional bait treatments (Buczkowski, 2016).

The goal of this study was to evaluate the efficacy of prey-baiting against Argentine ants in South African fynbos, a highly sensitive natural area where control efforts are rarely attempted for environmental reasons. The first part of the study evaluated prey-baiting in laboratory assays where *L. humile* colonies were presented with fipronil-treated termites within experimental arenas. The second objective tested the prey-baiting approach in natural areas invaded by *L. humile*. Taken together, the results contribute to the development of alternative management tools for invasive ants in sensitive environments.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site and termite collection

Field experiments were conducted at Helderberg Nature Reserve, Somerset West, Western Cape, South Africa (−34.06 S, 018.87 E). Helderberg Nature Reserve is a 398-hectare nature reserve located on the southern slopes of the Helderberg Mountains. Colonies of blackmound termites, *Amitermes hastatus* (Haviland), were collected within Helderberg Nature Reserve. *Amitermes hastatus* nests consist of small conical mounds (ca. 40 cm high) constructed of soil and organic matter. *Amitermes hastatus* was selected for the study because they appeared to be the most abundant termite within the reserve, and their mounds were present in areas invaded by Argentine ants. Other termite species, including harvester termites (*Hodotermitidae*) and nasute termites (*Nasutitermitinae*), were also present within the reserve, but were less common. The mounds were broken up using a rock hammer, and mound fragments containing termites were transported to the laboratory. Worker termites were extracted from the nesting material and placed *en masse* in plastic boxes with moist paper towels. The lids were replaced, and the boxes were kept at ambient temperature until the termites were used in experiments.

### 2.2 | *Linepithema humile* predation on termites—laboratory study

Experimental colonies (*n* = 4 per treatment) of *L. humile* were set up by aspirating 500 workers, 2 queens and 1 mg brood from stock colonies and transferring them into 25 × 30 × 9 cm high Fluon-coated plastic boxes containing an artificial nest consisting of a glass test tube (15 mm diameter × 150 mm long) half filled with water. The test tube was stoppered with a cork that contained a single hole (2 mm diameter) to allow entry. Each tube was wrapped in aluminium foil to keep it dark, and the aluminium sleeve could be pulled back to observe ant activity inside the tube. The ants were allowed to acclimate to the nest for 24 hr with food consisting of 25% sucrose solution. After acclimation, the treated termites were introduced outside the nest. To prepare treated termite prey for laboratory experiments, the termites were slightly chilled to slow their movements and were topically treated with 1 μl of 0.06% solution of Termidor SC (9.1% fipronil; BASF Corp., RTP, NC, USA), equivalent to 590 ng fipronil per termite. The insecticide was delivered using a microapplicator equipped with a 50-μl syringe (Hamilton Co., Reno, NV). Fipronil was selected because it is a broad-spectrum insecticide and is labelled for controlling both termites and ants. Fipronil is non-repellent to ants (Buczkowski, Scharf, Ratliff, & Bennett, 2005), and liquid baits containing fipronil are highly effective against ants, including *L. humile* (Hooper-Bui & Rust, 2000). The effect of prey number on *L. humile* mortality was tested by providing the experimental colonies with either 1 or 5 termite workers treated with fipronil. The behavioural interactions between ants and termites were observed continuously until all termites died. Subsequently, mortality in *L. humile* workers and queens was monitored hourly for the first 8 hr and then daily until all ants died. All experiments were performed at 25 ± 2°C, 60 ± 10% RH and 14:10 L:D cycle. Control tests ($n$ = 4) consisted of *L. humile* colonies provided with 1 termite sprayed with water alone.

### 2.3 | Control of *Linepithema humile* in invaded plots—field study

Field plots containing colonies of *L. humile* were established at Helderberg Nature Reserve. All plots were grassy areas directly adjacent to fynbos vegetation. The plots were 10 by 10 m and were separated by at least 25 m buffer zones. To estimate initial ant densities (day 0), the plots were sampled using note cards baited with a blend of canned tuna and honey (Buczkowski & Krushelnycky, 2012). Within each plot, the bait cards were placed along two transects, 10-m-long perpendicular lines forming a cross through the centre of each plot. Ten evenly spaced cards were used along each transect (20 baits per plot). The cards were placed on the ground and collected 1 hr after placement to record the presence of *L. humile*. Following census baiting, each 100 $m^2$ plot was subdivided into 1 $m^2$ sections, and each section was baited with 15 live termites (1,500 termites per plot) which had been topically sprayed with 0.06% fipronil. The 1,500 termites were placed in a plastic box and sprayed using a fine mister (atomizer). The atomizer was a 70-ml plastic bottle with a hand-pump sprayer (Specialty Bottle, Seattle, WA). Each pump from the atomizer delivers 150 μl of spray solution (±5%). Ten pumps from the atomizer were delivered for each box so all the termites were uniformly coated with a thin layer of the spray solution. Therefore, a total of 1.5 ml of fipronil solution was applied to each box. This is equivalent to 1-gallon solution per 1,000 square feet (40 ml per sq m), the recommended application rate for Termidor. The termites were held in the box for 1 hr, and symptomatic termites were scattered directly on the ground. Observations indicate that *L. humile* attacked the poisoned prey as soon as they were discovered and pulled them towards the nests. The efficacy of the prey-baiting treatment was examined on days 1, 3, 7, 14 and 21 using baited note cards as above. Six experimental plots and four control plots were established. Control plots were provided with termites treated with water. All assessments were performed in March 2017.

### 2.4 | Statistical analysis

A two-way repeated-measures Analysis of Variance was performed on two factors (i) the effect of fipronil and (ii) time to assess (a) the change in worker mortality over time in the laboratory after exposure to termites treated with fipronil, (b) the change in queen mortality over time in the laboratory after exposure to termites treated with fipronil, and (c) the change in worker abundance over time under field conditions after treatment with fipronil laced termites. The Greenhouse-Geisser correction was applied due to violations of the assumption of sphericity (Mauchly's test) and equality of variances (Levene's test). This test recalculates new degrees of freedom to obtain a valid $F$-ratio. Tukey's HSD test was used for post hoc pairwise comparisons, and parameter estimates showed changes in worker abundances relative to the reference group, set as control, for both laboratory and field colonies. Average per cent worker and queen mortality curves were generated to visually show these changes. Statistical significance was set at $\alpha$ = .05. All statistical analyses were performed using Statistica 13.2 (Statistica 2017).

## 3 | RESULTS

### 3.1 | *Linepithema humile* predation on termites—laboratory study

Termites placed within *L. humile* colonies were symptomatic and exhibited classical signs of insecticide poisoning including twitching, erratic walking and inability to maintain an upright stance. The termites were readily attacked by foraging workers and carried directly to the nest. Several ants typically attacked a single termite which most likely facilitated the transfer of fipronil from the termites to the ants through direct contact. The termites were completely consumed, and no termite remains could be found in ant nests 24 hr after the termites were introduced. Mortality in *L. humile* was relatively quick and all colonies died within 4 days of being provisioned with fipronil-treated termites (Figure 1a,b). In colonies provided with a single termite mortality in the workers reached 73 ± 5% in 24 hr and 100% mortality was achieved within 96 hr (Figure 1a). Worker mortality increased significantly with increasing time of exposure (repeated-measures ANOVA, time × treatment: $F_{(3.94,36)} = 26.17$, $p < .0001$, $\eta^2 = 0.85$, $\varepsilon = 0.49$, Table 1). Twenty-four hours after exposure to termites, mortality in workers in the 1-termite treatment ($B = 71.45$, $SE = 5.82$, $t = 12.28$, $p < .0001$) and the 5-termite treatment ($B = 73.25$, $SE = 5.82$, $t = 12.58$, $p < .0001$) was significantly

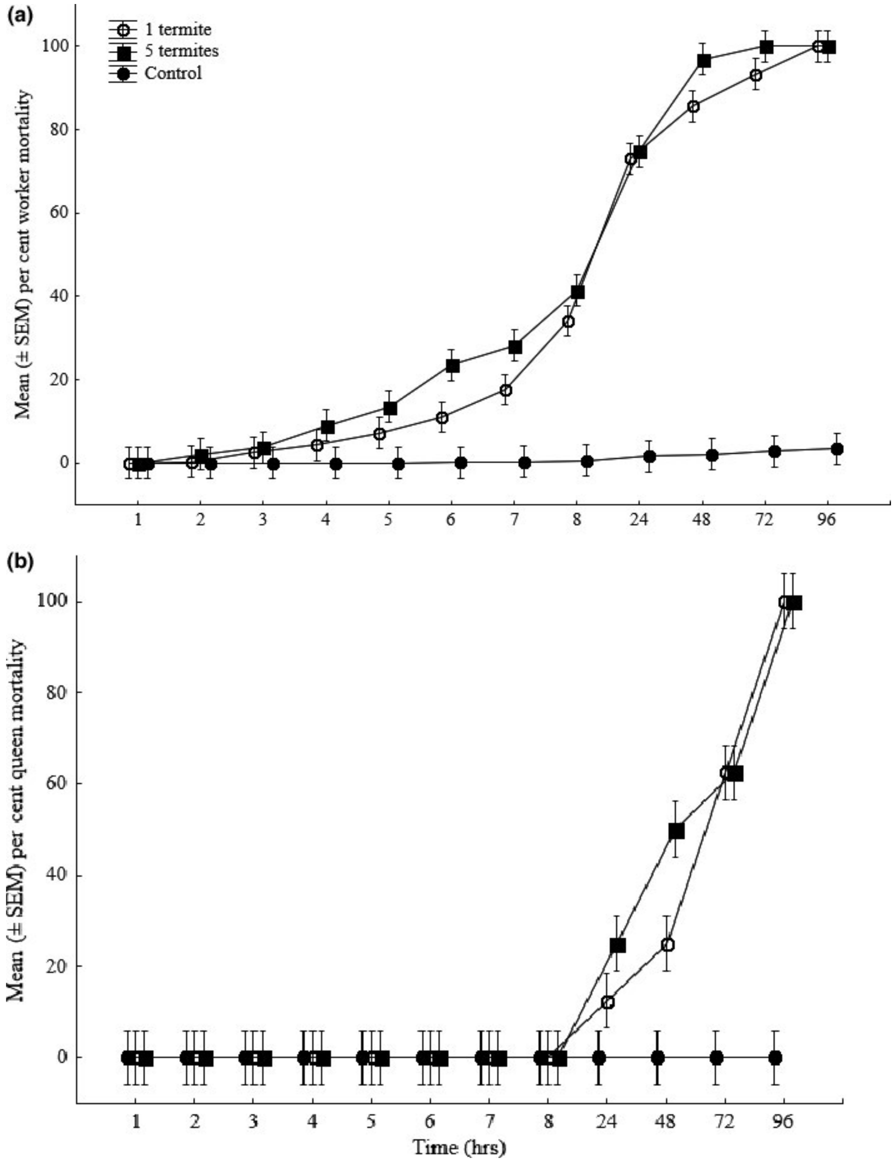


**FIGURE 1** Mean (±*SEM*) cumulative per cent mortality in (a) workers and (b) queens in laboratory colonies of *Linepithema humile* provided with one or five donor termites

higher relative to the control. Similar increased mortality for both treatment groups, relative to the control group, was obtained for the other assessment times. Mortality in the queens was initially delayed, and no queens died during the first 8 hr (Figure 1b); however, queen mortality in both treatment groups differed from the control group from 24 hr and all queens in both treatment groups were killed by 96 hr (repeated-measures ANOVA, time × treatment: $F_{(4,36)} = 10.20$, $p < .001$, Table 1).

## 3.2 | *Linepithema humile* predation on termites—field study

Argentine ants were the dominant species in all experimental plots and were present in relatively high densities. The ants nested in soil and occupied multiple nests connected by a network of above-ground trails. The bait scatter approach appeared to work well because it placed prey items in close proximity to multiple nests and foraging trails. Throughout the study, <0.5% of bait cards were discovered by native ant species. Observations indicate that Argentine ants readily attacked the termite prey and carried them towards their subterranean nests. No other ants or non-target animals were ever observed collecting the termites, most likely because they were almost completely absent from the plots. Furthermore, Argentine ants removed the majority of termites from the soil surface within approximately 30 min of application further minimizing the risk of non-target exposure.

The application of fipronil-treated termites reduced *L. humile* abundance over time relative to the untreated control plots (Figure 2). At 21 days after the initial treatment, the abundance of *L. humile* declined by 98 ± 5% within the treated plots. The number of *L. humile* detected over time differed significantly between the treated and the control plots (repeated-measures ANOVA,

**TABLE 1** Mean cumulative per cent mortality (±*SEM*) in *Linepithema humile* workers and queens exposed to fipronil-treated *Amitermes hastatus* termites

| Ant caste | Ant: termite ratio | Time (hr) 2 | 4 | 6 | 8 | 24 | 48 | 72 | 96 |
|---|---|---|---|---|---|---|---|---|---|
| Workers | One donor termite | 0 ± 0 a | 4 ± 1 a | 11 ± 1 a | 34 ± 4 a | 73 ± 5 a | 86 ± 3 a | 93 ± 3 a | 100 ± 0 a |
| | Five donor termites | 2 ± 1 a | 9 ± 2 a | 24 ± 2 b | 41 ± 4 a | 75 ± 5 a | 97 ± 2 b | 100 ± 0 a | 100 ± 0 a |
| | Control | 0 ± 0 a | 0 ± 0 a | 0 ± 0 c | 1 ± 0 b | 2 ± 0 c | 2 ± 1 c | 3 ± 1 b | 4 ± 1 b |
| Queens | One donor termite | 0 ± 0 a | 0 ± 0 a | 0 ± 0 a | 0 ± 0 a | 13 ± 13 a | 25 ± 14 a | 63 ± 13 a | 100 ± 0 a |
| | Five donor termites | 0 ± 0 a | 0 ± 0 a | 0 ± 0 a | 0 ± 0 a | 25 ± 14 a | 50 ± 20 a | 63 ± 13 a | 100 ± 0 a |
| | Control | 0 ± 0 a | 0 ± 0 a | 0 ± 0 a | 0 ± 0 a | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b |

Within each ant caste, means followed by the same letter are not significantly different ($p \le .05$).

time × treatment: $F_{(4.72,35)} = 10.00$, $p < .001$, $\varepsilon = 0.34$, $\eta^2 = 0.94$). At the start of the treatment, no difference in ant abundance was found between plots in the control and fipronil-treated plots ($B = 317.17$, $SE = 421.35$, $t = 0.75$, $p = .48$, Figure 2), but a significant decline in the abundance of ants in the fipronil-treated plots was observed from 24 hr ($B = -1{,}048.9$, $SE = 178.05$, $t = -5.78$, $p < .0001$, Figure 2). The number of ants in the treated plots differed significantly from controls at each assessment time after treatment (Figure 2).

## 4 | DISCUSSION

Results of the current study demonstrate that prey-baiting is highly effective against Argentine ants in both laboratory and field trials. Argentine ants are classified as omnivores (Holway et al., 2002)—they rely heavily on carbohydrate-rich plant and insect exudates, but are also opportunistic scavengers and aggressively attack a variety

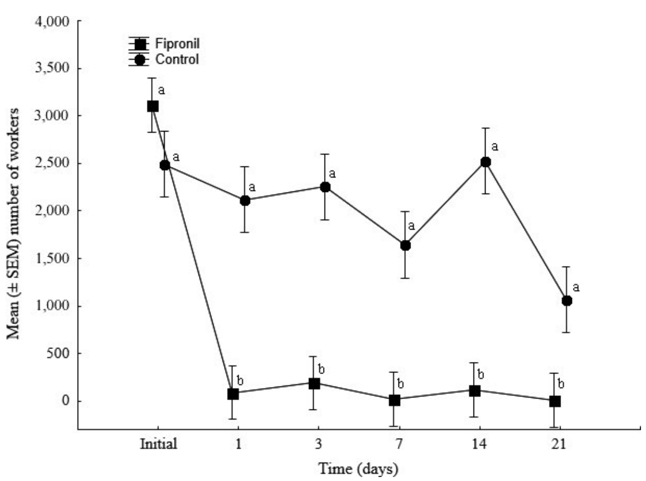


**FIGURE 2** Mean (±*SEM*) number of *Linepithema humile* workers detected within field plots treated with fipronil-treated or control termites. Letters indicate pairwise differences in ant abundance at each assessment time between fipronil-treated and control plots

of insects present within their foraging territories. Argentine ants readily attacked fipronil-treated termites and carried them back to the nest(s) where they were subsequently dismembered and consumed. Mortality in the ants was likely due to a combination of contact toxicity (handling fipronil-treated termites) and oral toxicity (feeding on fipronil-treated termites). Previous studies demonstrated that fipronil is highly toxic to Argentine ants by ingestion (Hayasaka et al., 2015; Wiltz, Suiter, & Gardner, 2009) and contact (Choe & Rust, 2008, Soeprono and Rust 2004a). Observations indicate that the ants spent a significant amount of time attacking and killing the termites, collectively dragging them to the nests, and dismembering the termite bodies. Such behaviours most likely facilitated the transfer of fipronil from the termites to the ants. However, mortality in the queens was initially delayed. The factor(s) responsible for the delay are not clear. A previous study reported a similar result whereby mortality in Argentine ant queens exposed to fipronil bait was delayed and dependent on the starvation level (Mathieson, Toft, & Lester, 2012). In the current study, multiple factors could have played a role. One potential factor is the difference in prey handling between workers and queens. Unlike workers, the queens were never observed attacking the termites. Consequently, contact toxicity most likely played a minimal role in queen mortality. Additionally, queens are much larger relative to workers, and a higher dose of fipronil might be required to kill queens.

The development of novel and effective techniques to eradicate populations of invasive ants is essential for biodiversity conservation, human welfare and the preservation of sensitive ecosystems worldwide (Hoffmann et al., 2016). Invasive ant management has evolved over the last century, and new active ingredients and innovative delivery tools have emerged to provide safer and more effective means of controlling invasive ants (Hoffmann, Abbott, & Davis, 2010; Williams, 2003). The shift from broadcast spraying of contact insecticides to the use of baits resulted in greater efficacy, safer application, reduced environmental pollution and fewer non-target impacts. However, the scope and success of ant eradications have been limited, and there is a continued need to develop more effective

**TABLE 2** Comparison of different commercial and experimental approaches for managing invasive ants

| Control method | Product | Recommended application rate | Amount of fipronil applied per 100 sq m (g) | Fold increase in fipronil use relative to prey-baiting |
|---|---|---|---|---|
| Granular application | Top Choice (Bayer) 0.0143% fipronil | 1 kg bait/100 sq m | 14.3 | 16,158 |
| Liquid spray application | Termidor SC (BASF) 0.06% fipronil | 407 ml/100 sq m | 2.34 | 2,644 |
| Bait crystals (hydrogels) | Polyacrylamide crystals saturated with 0.001% fipronil in sugar water | 200 ml bait/100 sq m | 0.20 | 226 |
| Prey-baiting | Live termites sprayed with 1.5 ml of 0.06% fipronil | 1,500 termites/100 sq m | 0.000885 | 1 |

management tools (Hoffmann et al., 2016). Prey-baiting is a novel strategy for invasive ant management that integrates natural history of the target species into the management approach (Buczkowski, 2016, 2017). Prey-baiting exploits the predatory feeding habits of many invasive ants and uses live, insecticide-treated prey to deliver the toxicant to the target species. Relative to traditional baits, prey-baiting offers greater bait selectivity and consequently greater target specificity (Buczkowski, 2017). Additionally, prey-baiting uses significantly less toxicant relative to traditional bait treatments and provides environmental benefits with regard to pesticide residues in sensitive environments (Buczkowski, 2016).

Relative to traditional liquid baits, which are ingested without the need to process the bait, prey-baiting increases prey handling time which in turn facilitates the spread of the toxicant among colony members. The potential for fipronil to be horizontally transferred among colony members may also contribute to the effectiveness of the prey-baiting approach. Fipronil is readily transferred by physical contact in Argentine ant colonies (Soeprono & Rust, 2004a). In laboratory studies, donor ants exposed to fipronil-treated sand were placed in a colony of untreated recipient ants. Fipronil was effectively transferred from treated donors to untreated recipients: 10 ants exposed to fipronil for 1 min transferred enough fipronil to nearly eliminate 200 workers and worker mortality reached 97% within 6 days (Soeprono & Rust, 2004b). Necrophoresis (carrying of dead nestmates to refuse piles) was the main mechanism responsible for the transfer of fipronil. Other social behaviours, such as mutual grooming, antennal contacts and trophallaxis, may have also contributed to the horizontal transfer of fipronil among nestmates. Additionally, fipronil is highly soluble in lipids such as those present in the wax layer of the insect cuticle which might contribute to its efficacy and potential for horizontal transfer. The combination of delayed toxicity, non-repellency, high contact toxicity and high potential for horizontal transfer contribute to fipronil's efficacy against social insects (Soeprono & Rust, 2004a). These properties also make fipronil uniquely suitable for use in prey-baiting.

The laboratory study revealed that a single termite treated with 590 ng fipronil is capable of killing at least 500 Argentine ant workers and that the poisoned workers subsequently transfer a lethal dose to the queens. In the field study, 1,500 termites were scattered within each 100 $m^2$ plot, enough to kill at least 750,000 Argentine ants. The actual number of Argentine ants within the experimental plots was unknown, but results indicate that 15 termites per square metre are sufficient to provide 98%–100% control. Furthermore, this level of control can be achieved despite a constant influx of new ants into the treated plots from the surrounding untreated areas. Similar results were obtained in experiments that evaluated prey-baiting against Asian needle ants, *Brachyponera chinenesis* (Buczkowski, 2016). A single termite (*Reticulitermes flavipes*) carrying 10 ng fipronil killed 100 ants in laboratory tests, and >98% control was achieved in a field test within 28 days. These results demonstrate that fipronil is effective against invasive ants in ultralow amounts and highly suitable for the prey-baiting approach.

Fipronil is a broad-spectrum insecticide and poses a potential threat to non-target invertebrates. However, fipronil is typically used in extremely low concentrations, is not persistent, and has low soil mobility (Gunasekara, Truong, Goh, Spurlock, & Tjeerdema, 2007). The environmental fate of fipronil and its metabolites were tracked in an ant eradication study on Christmas Island, and no residues were detected in the soil 1 week after baiting throughout multiple baiting events (Marr, O'Dowd, & Green, 2003). In another study, non-target impacts were monitored during a large-scale yellow crazy ant control programme using fipronil bait (Stork, Kitching, Davis, & Abbott, 2014). No significant difference was detected in the abundance and diversity of arthropods between treated and untreated plots. Additionally, the ecological impact of prey-baiting was tested in a study that utilized fipronil-treated termites to control Asian needle ants (Buczkowski, 2017). Results demonstrated that prey-baiting is target-specific with detrimental effects against the target species and negligible effects on native ants (Buczkowski, 2017).

In the current study, Argentine ants dominated the experimental plots and native ants were rarely present. However, two native species, *Anoplolepis custodiens* and *Lepisiota capensis*, were detected on bait monitoring stations in two experimental plots. The native ants were most likely not affected by prey-baiting because they were present in very low densities relative to Argentine ants and Argentine ants dominated and quickly retrieved the termite prey. Argentine ants are able to monopolize resources in interactions with native ants (Rowles & O'Dowd, 2007) and become the primary target of toxic baits when they aggressively outcompete native ants from toxic baits through efficient interference competition (Buczkowski & Bennett, 2008).

The prey-baiting approach evaluated in this study offers a safer and more environmentally friendly alternative to traditional insecticide treatments such as spray and granular applications. Such products are typically applied at relatively high rates resulting in environmental persistence and non-target effects (Table 2). Prey-baiting has the potential to significantly reduce the amount of active ingredient placed in the environment, while providing a high level of efficacy. In the current study, 1,500 termites were used per 100 $m^2$, equivalent to approximately 0.00089 g fipronil per plot (Table 2). In contrast, a typical spray application would require 2.34 g fipronil (2,644 times higher), and a granular application would require 14.3 g fipronil (16,158 times higher). Prey-baiting also offers environmental benefits with regard to pesticide residues in ecologically sensitive environments where invasive ants pose a threat to native organisms and must be carefully managed to avoid non-target effects (e.g., Allen, Epperson, & Garmestani, 2004; Gerlach, 2004; Plentovich, Hebshi, & Conant, 2009). Prey-baiting is also more targeted because the toxicant is applied directly to the prey which are harvested and taken directly to the nests. The prey are removed from the soil surface where they could be retrieved by non-targets and placed in close proximity to ant colonies. In contrast, broadcast spray treatments are applied to soil surface and do not directly affect Argentine ant nests which are subterranean.

In summary, prey-baiting appears to be a practical and effective method of delivering toxicants to Argentine ants. Relative to commercially manufactured baits and other control methods, prey-baiting uses significantly less toxicant and is therefore ideally suited for ant management in conservation areas. The field trial demonstrated immediate and sustained control of Argentine ants and suggests that prey-baiting could be up-scaled to larger infestations. Furthermore, prey-baiting should be adapted to control or eliminate populations of other invasive ants.

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## AUTHOR CONTRIBUTION

GB conceived research. GB, NPM and TCW conducted experiments. GB, NPM and TCW contributed material. GB and NPM analysed data and conducted statistical analyses. GB wrote the manuscript. GB and TCW secured funding. All authors read and approved the manuscript.

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