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The Trojan horse approach for managing invasive ants: a study with Asian needle ants, *Pachycondyla chinensis*

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Abstract Ants are among the most ecologically and economically significant biological invaders and are notoriously difficult to eradicate once established. Invasive ants are typically managed with toxic baits which are often unattractive to the target species, toxic to non-targets, and environmentally persistent. The current study evaluated a novel Trojan horse approach for managing invasive ants in natural habitats based on the use of poisoned prey. Eastern subterranean termites (*Reticulitermes flavipes*) were topically exposed to fipronil and presented to Asian needle ants (*Pachycondyla chinensis*) which are a significant invader in natural and disturbed habitats in the eastern United States. In laboratory assays, *P. chinensis* colonies were offered fipronil-treated termites within experimental arenas. The termites were readily attacked and consumed and results demonstrate that a single termite exposed to 25 ppm fipronil for 1 h is capable of killing 100 *P. chinensis* workers in 9 h. To evaluate population effects, field studies were conducted in forested areas invaded by *P. chinensis*. Fipronil-treated termites scattered on the forest floor provided rapid control of *P. chinensis* and ant densities throughout the treated plots declined by 98 ± 5 % within 28 days. I demonstrate that the poison baiting approach based on fipronil-treated termite prey is highly effective against *P. chinensis* and may offer an effective alternative to traditional bait treatments against other invasive ants, especially those with predatory and generalist feeding habits. Furthermore, I demonstrate that the poison baiting approach offers environmental benefits by delivering substantially less toxicant to the environment relative to current control methods which rely on commercial bait formulations. In summary, the poison baiting approach evaluated in this study appears highly suitable for controlling invasive ants and should be further tested against other invasive ants.

Keywords Asian needle ant · Bait · Control · Fipronil · *Pachycondyla chinensis* · Predation · Predator–prey interactions · *Reticulitermes flavipes* · Termite

Introduction

Invasive ants are among the most globally damaging invasive species with significant economic, environmental, and social impacts (Lowe et al. 2000; Holway et al. 2002; Lach and Hooper-Bui 2010). Once established, they are notoriously difficult to eradicate especially when invading propagules elude early detection and populations reach high densities over large geographic areas (Silverman and Brightwell 2008; Buczkowski and Krushelnycky 2012; Hoffmann et al. 2010). The spread and impact of invasive

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ants is often limited using chemical management tools, predominantly baits and residual sprays. Toxic baits are especially popular in controlling invasive ants and have been used to control a wide range of species (e.g. Causton et al. 2005; Daane et al. 2008; Drees et al. 2013; Buczkowski et al. 2014). Despite some successes with toxic baits (e.g. Lester and Keall 2005; Hoffmann 2010), baits suffer a number of disadvantages that limit their use. This includes relatively short life span under field conditions, susceptibility to environmental factors, ecological contamination, lack of effective dispensers, and non-target effects. Toxic baits must be used with caution in area-wide treatments in natural areas where native ant conservation is a concern. This is because toxic baits are typically attractive to a wide range of ants and may kill nontarget organisms they are designed to protect. Non-target effects can be limited by offering granular baits with particle sizes that are optimal for the target species, therefore increasing bait selectivity (Hooper-Bui et al. 2002). Bait selectivity may also be achieved by utilizing bait formulations that most closely mimic the natural food sources of the target species. For example, Argentine ants feed largely on hemipteran honeydew and liquid baits with sucrose most closely resemble this natural food source and are the most attractive (Silverman and Brightwell 2008). Finally, bait selectivity may be achieved indirectly through reliance on the target ant's superior interference and exploitative ability. For example, Buczkowski and Bennett (2008) demonstrated that Argentine ants outcompete native ants at toxic baits due to their superior interference and exploitative competition ability and consequently suffer higher mortality by becoming the primary target of baits.

The Asian needle ant, *Pachycondyla chinensis* (Emery), is an invasive ant species introduced into the United States from Japan in the early 1930s (Smith 1934). Following the initial introduction, the species remained largely inconspicuous for several decades (McGown 2009). Recently, however, *P. chinensis* have become widespread in parts of the southeastern US and are now a common pest in urban and natural habitats (Guenard and Dunn 2010). In mature temperate forests, *P. chinensis* cause a strong decline in native ant abundance (Guenard and Dunn 2010) and disrupt ant-seed dispersal mutualisms by displacing native keystone ant species (Rodriguez-Cabal et al. 2012). In parts of North Carolina, USA, *P. chinensis* are displacing *L. humile* by expanding their colonies early in the season (Spicer-Rice and Silverman 2013). Furthermore, recent predictive modeling demonstrates that climate change is going to significantly increase the global spread of *P. chinensis* by increasing the amount of habitat suitable to their invasion by 65 % worldwide (Bertelsmeier et al. 2013). Despite the apparent explosive increase in *P. chinensis*' range and ecological impact, little is known about effective management approaches for this species. Spicer-Rice et al. (2012) demonstrated effective control using a commercial bait formulation, but other management approaches have not been investigated. The lack of effective management approaches may be related, at least in part, to the biology of *P. chinensis* which is unique among invasive ants. First, while most invasive ants utilize carbohydrate-rich food sources consisting of floral nectar and hemipteran honeydew (Holway et al. 2002), *P. chinensis* is a predatory ant and a termite specialist (Bednar and Silverman 2011). There is no evidence that *P. chinensis* consumes nectar or hemipteran honeydew which precludes the use of liquid baits for its control. Second, most invasive ants use mass recruitment via trail pheromones to collect food or toxic baits during management attempts. In contrast, no trail pheromones have been detected in *P. chinensis*. Instead, *P. chinensis* employs a unique yet relatively slow recruitment process called tandem carrying whereby foraging workers carry nestmates from the nest to the food source which is subsequently retrieved (Guenard and Silverman 2011). Finally, unlike colonies of many invasive ants which dominate urbanized and disturbed habitats, colonies of *P. chinensis* have the unique ability to invade habitats in undisturbed hardwood forests. These factors complicate management efforts for *P. chinensis* and warrant the search for novel management tools and approaches to combat this invasive species.

Given that *P. chinensis* is an ant that preys on termites, I examined the potential of live termite prey to deliver insecticide, and thereby cause ant colony demise. The first part of the study evaluated predation in laboratory assays where *P. chinensis* colonies were presented with insecticide-treated termites within experimental arenas. The second objective tested the poison baiting approach in natural areas invaded by *P. chinensis*. The broader goal of this project was to perform a demonstration study utilizing *P. chinensis* as a model organism to evaluate the Trojan horse approach for managing invasive ants and possibly other invasive taxa.

Materials and methods

Collection and preparation of fipronil-treated termites

Colonies of eastern subterranean termites, *Reticulitermes flavipes*, were collected from cardboard-baited traps buried in areas known to harbor termite colonies (Buczkowski et al. 2007). The termites were brought into the laboratory and allowed to migrate into plastic containers with cellulose powder, moistened pine wood, and laboratory paper towels provided as food and harborage. Colonies were maintained at 25–27 °C, >80 % RH, and in constant darkness. Fifth through seventh instar workers were used in all assays. To prepare the donor termites for laboratory and field studies, construction sand (Quikrete Play Sand, Atlanta, GA) was autoclaved for 1 h at 120 °C and dried. To prepare 25 ppm fipronil-treated sand, 138 μL of Termidor SC (9.1 % fipronil, BASF Corp., RTP, NC) was dissolved in 100 mL water and thoroughly mixed with 500 g sand in a sealed Ziploc bag. The 25 ppm dose was selected based on Saran and Rust (2007) who reported that individual termites pick up approximately 10 ng fipronil when exposed to sand treated with 25 ppm fipronil. To prepare the donors, 150 g of the mixture was placed in a Petri dish (14 cm diameter) and approximately 250 termites were added. Termite mortality is a function of fipronil concentration and time spent on the treated substrate. The goal was to produce donor termites that had a sufficiently high amount of fipronil, but were still alive (either symptomatic or asymptomatic) when presented to the ants. Preliminary tests showed that termites exposed to 25 ppm fipronil-treated sand remained alive for approximately 60 min. Therefore, termites exposed for 60 min were used in all subsequent laboratory and field experiments.

Pachycondyla chinensis predation on termites: laboratory study

Colonies of *P. chinensis*, were collected from rotting logs in wooded areas at Tanglewood Park Golf Course in Clemmons, NC (36.00°N, −80.40°W) and Bermuda Run Country Club in Bermuda Run, NC (36.00°N, −80.42°W). The colonies were transported to the laboratory and experimental colonies were set up by aspirating 100 workers and several brood from stock colonies and transferring them into artificial nests consisting of a glass test tube (15 mm diameter × 150 mm long) half filled with moist soil. The test tube was stoppered with a cork that contained a single hole (2 mm diameter) to allow entry. Each tube was wrapped in aluminum foil to keep it dark and the aluminum sleeve could be pulled back to observe ant activity inside the tube. Experimental colonies were placed in 25 × 30 × 9 cm high Fluon-coated plastic boxes and the ants were allowed to acclimate to the nest for 24 h without food. After acclimation, the experimental prey (fipronil-treated termites) were introduced outside the nest. The effect of prey number on *P. chinensis* colony mortality was tested by providing the experimental colonies with either 1, 5, or 10 termite workers treated with fipronil as above. Each treatment was replicated six times. The behavioral interactions between ants and termites were observed continuously until all termites died. Subsequently, mortality in *P. chinensis* workers was monitored hourly until all ants died. All experiments were performed at 25 ± 2 °C, 60 ± 10 % RH, and 14:10 L:D cycle. Control tests (n = 6) consisted of *P. chinensis* colonies provided with 1 termite exposed to sand treated with water alone.

Pachycondyla chinensis predation on termites: field study

Field plots containing colonies of *P. chinensis* were established at Tanglewood Park Golf Course and Bermuda Run Country Club as above. All plots at both locations were golf course roughs and wooded out-of-bounds areas surrounding the fairways. At each site, five experimental plots and three control plots were established. All plots were 6 by 6 m and test plots were separated by at least 25 m buffer zones. To estimate the initial ant densities (day 0) the plots were sampled on a 2 by 2 m grid (9 sub-plots; 16 baits per plot) using note cards baited with a blend of canned tuna and corn syrup (Buczkowski and Krushelnycky 2012). The note cards were placed on the ground and collected 2 h after placement to record the presence of *P. chinensis*. Following census baiting, each 36 m^2 plot was subdivided into 1 m^2 sections and each section was baited with 15 live termites (540 termites per plot) which had been exposed to fipronil-treated sand for 60 min as above. Within each 1 m^2 section, the termites were scattered on the ground, concentrating on any logs that may have been present within the plots. Observations indicate that *P. chinensis* attacked and retrieved some of the poisoned prey, but the ultimate fate of all the termites is unknown and they may have been retrieved by other scavengers. Spicer-Rice et al. (2012) evaluated clumped and scattered applications of hydramethylnon baits for *P. chinensis* management and reported no difference between the two application methods. The scattered approach was used in the present study, especially since the clumped approach would not have worked with live prey which are able to disperse and move away from the point of application. The efficacy of the treatments was examined on days 1, 3, 7, 14, and 28 using baited note cards as above. All tests were performed in April–May 2015.

Statistical analysis

For the laboratory assay, a multivariate repeated measures test was used to examine the effect of treatment (number of fipronil-treated termites introduced into ant colonies), time, and the interaction on ant mortality. This was followed by univariate ANOVA to examine variation at each time point. Comparisons among treatments or among exposure times consisted of ANOVA tests on mean cumulative percent mortality followed by Tukey's HSD test to test for significant differences among treatment means on each date. The level of significance was set at $\alpha = 0.05$. All statistical analyses were performed using Statistica 12.6 (Statistica 2014).

Results

Pachycondyla chinensis predation on termites: laboratory results

All termites were killed on first contact with *P. chinensis* (Fig. 1a, b) and immediately taken back to the nest. Behavioral observations revealed that *P. chinensis* have a long-range ability to detect the termites. The ants became visibly excited when a termite was present within approximately 2 cm away.

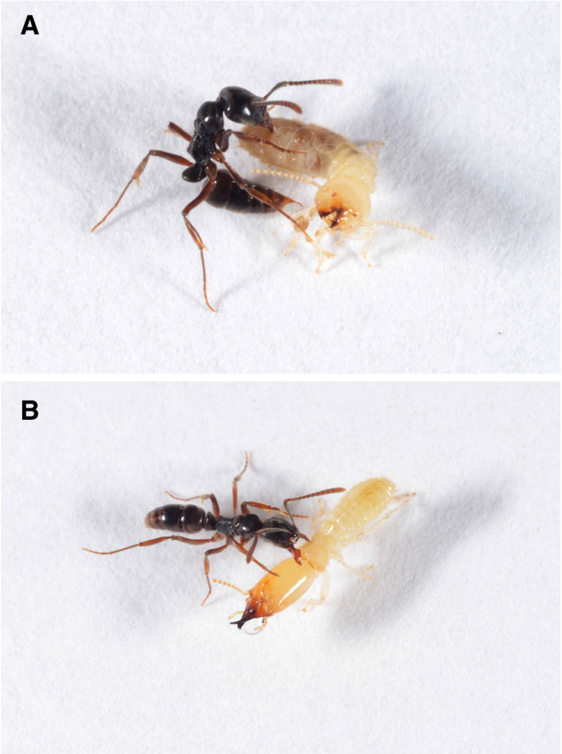

Fig. 1 Asian needle ant (*Pachycondyla chinensis*) attacking eastern subterranean termite (*Reticulitermes flavipes*) **a** workers and **b** soldiers. Despite sclerotized mandibles in both workers and soldiers, the termites appear completely defenseless and are easily subdued by ant venom

The ants exhibited fast, erratic running and directed movement toward the termite. This suggests that termites emit volatile chemicals that are detected by *P. chinensis* may help the ants locate their prey. Alternatively, *P. chinensis* may use visual cues in locating termites or a combination of visual and chemical cues.

Mortality in *P. chinensis* colonies provisioned with fipronil-treated termites was relatively quick and all ants died within 9 h of being provided with the termites (Table 1; Fig. 2). Complete mortality was achieved in all *P. chinensis* colonies regardless of the number of termites introduced into the colonies (Table 1; Fig. 2). The first symptomatic *P. chinensis* workers appeared approximately 2 h after being presented with the termites. Symptoms of poisoning included erratic walking, inability to maintain an upright stance, and twitching. Symptomatic workers typically died within 1–2 h. At the end of the test (9 h), all ant nests were inspected to determine the fate of the termites. The majority of the termites introduced to the

Table 1 Cumulative mean percent mortality (±SE) in *P. chinensis* colonies exposed to fipronil-treated *R. flavipes* termites

	Time (h)								
	1	2	3	4	5	6	7	8	9
1 Donor termite	0 ± 0 ab	0 ± 0 ab	4 ± 3 abc	15 ± 6 def	39 ± 11 g	66 ± 8 h	82 ± 9 ijk	92 ± 8 lm	100 ± 0 m
5 Donor termites	0 ± 0 ab	1 ± 1 a	12 ± 3 bcd	25 ± 8 ef	73 ± 14 hi	87 ± 8 jkl	94 ± 5 lm	100 ± 0 m	100 ± 0 m
10 Donor termites	0 ± 0 ab	1 ± 1 a	15 ± 5 cde	26 ± 6 f	80 ± 10 ij	91 ± 8 klm	97 ± 3 lm	100 ± 0 m	100 ± 0 m
Control	0 ± 0 ab	0 ± 0 ab	0 ± 0 ab	0 ± 0 ab	0 ± 0 ab	0 ± 0 ab	0 ± 0 ab	0 ± 0 ab	0 ± 0 ab

Means followed by the same letter are not significantly different ($P \leq 0.05$) based on Tukey's HSD test

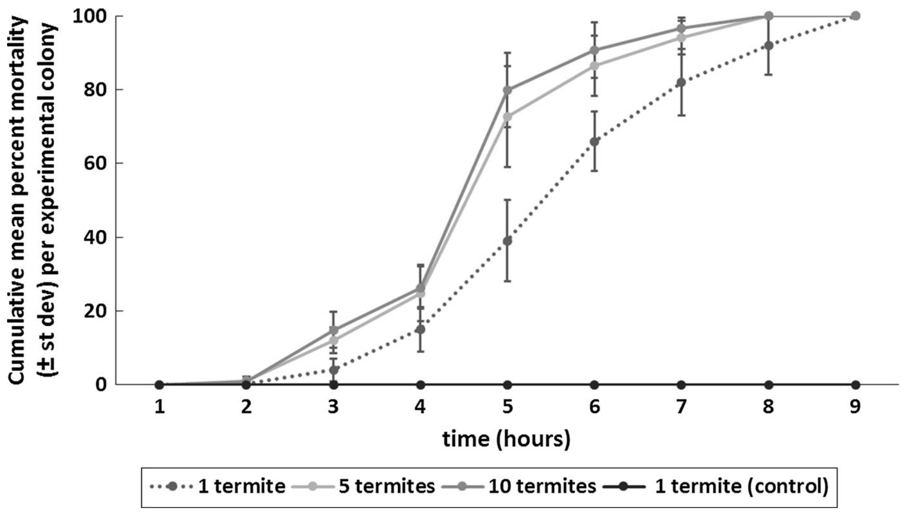

Fig. 2 The mean (±SD) cumulative percent mortality in laboratory colonies of *P. chinensis* provided with fipronil-treated termites

colonies were dismembered (e.g. head separated from the body) and appeared to be partially consumed. However, it appears that mortality in *P. chinensis* was mostly due to topical toxicity (handling of fipronil-treated termites) rather than oral toxicity (ingestion of termites). Behaviors that facilitated mortality in *P. chinensis* included fighting with the termites, carrying paralyzed termites back to the nest, and dismembering dead termites.

A single termite exposed to 25 ppm fipronil was capable of killing 100 *P. chinensis* workers (Table 1). The overall effect of treatment (number of fipronil-treated termites introduced into ant colonies) was significant ($F = 540.3$, $df = 3$, $P = 0.001$). Among the different treatments, no significant difference in *P. chinensis* mortality was detected in colonies provided with 5 or 10 termites ($F = 2.02$, $df = 3$, $P = 0.142$). While a single treated termite caused complete mortality in 9 h, the rate of mortality was significantly increased when the colonies were provided with 5 termites ($F = 28.03$, $df = 3$, $P < 0.0001$) or 10 termites ($F = 23.50$, $df = 3$, $P < 0.0001$). No ants died in any of the control tests.

Pachycondyla chinensis predation on termites: field study

The application of fipronil-treated termites reduced *P. chinensis* abundance over time relative to the untreated control plots (Fig. 3). At 28 days after the initial treatment, the abundance of *P. chinensis* declined by 98 ± 5 % within the treated plots (Table 2). The number of *P. chinensis* detected over time differed significantly across treatments (time x

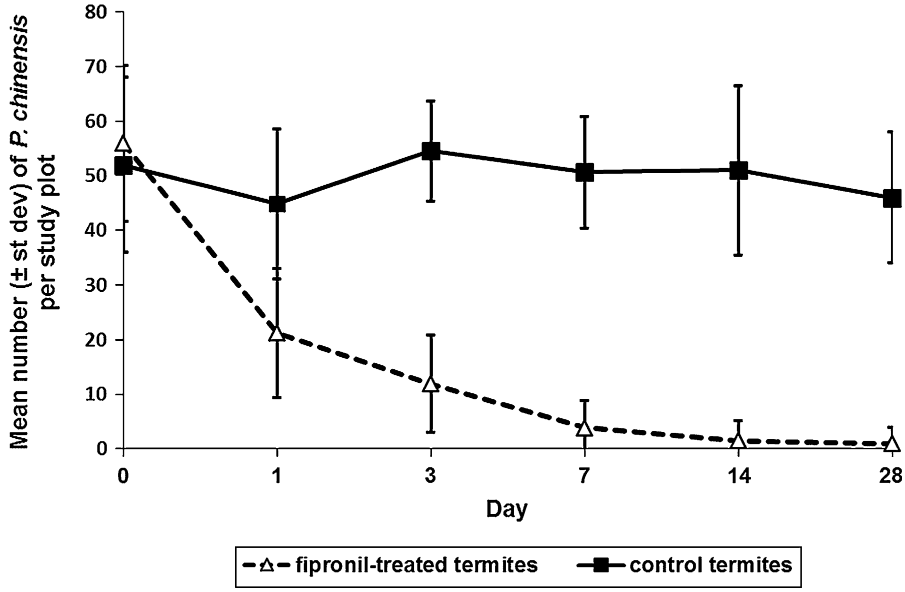

Fig. 3 The mean (±SD) number of *P. chinensis* workers detected in field plots provided with fipronil-treated termites

Table 2 Mean percent change (±SE) in the total number of *P. chinensis* workers detected at bait stations within the experimental plots

Treatment	Time (days)				
	1	3	7	14	28
Fipronil-treated termites	−62 ± 19 b	−80 ± 13 bc	−94 ± 8 c	−97 ± 6 c	−98 ± 5 c
Control	−8 ± 32 a	+18 ± 83 a	+6 ± 56 a	+7 ± 53 a	−8 ± 15 a

Means followed by the same letter are not significantly different ($P \leq 0.05$) based on Tukey's HSD test

treatment: $F = 25.91$, $df = 5$, $P < 0.0001$). Ant activity was completely absent in 6 out of 6 Tanglewood Park sites and 4 out of 6 Bermuda Run sites.

Discussion

The current study evaluated a novel Trojan horse approach for managing invasive ants in natural habitats and demonstrated that the use of poisoned termite prey provided rapid control of *P. chinensis*. The novelty of the project lies in using live termite prey for delivering the toxicant to the target species. Previous management approaches for invasive ants have only utilized commercial bait formulations (Hoffmann et al. 2010) and the feasibility and efficacy of other approaches has not been investigated.

In laboratory studies, fipronil-treated termites were readily attacked by *P. chinensis* and envenomated termites were carried back to the nest where they were subsequently dismembered and consumed. Fipronil is non-repellent to ants (Buczkowski et al. 2005) and liquid baits containing fipronil are highly effective against other invasive ants, including Argentine ants (Hooper-Bui and Rust 2000). Furthermore, laboratory tests demonstrated that a single termite exposed to 25 ppm fipronil is capable of killing 100 *P. chinensis* workers within approximately 9 h. Fipronil is readily transferred among ants (Soeprono and Rust 2004) which may explain its effectiveness against *P. chinensis*. However, the relative roles of oral toxicity (feeding on poisoned termites) versus topical toxicity (handling poisoned termites) remains unclear. It is also unclear whether the ants acquired fipronil directly by handling the poisoned termites or indirectly by interacting with nestmates that had previously handled poisoned termites. Ant behaviors such as mutual grooming, trophallaxis, and necrophoresis have been shown to be important factors in the transfer of fipronil within ant colonies (Soeprono and Rust 2004).

Overall, the results suggest that fipronil is effective against *P. chinensis* in ultralow amounts and therefore appears to be highly suitable for the poison-baiting approach.

The development of effective techniques to eradicate populations of invasive ants is crucial to the conservation of native biodiversity. One of the main advantages of the baiting approach developed in this study is that it may offer greater bait selectivity and consequently greater target specificity. Traditional baits are typically non-selective and attract a wide range of non-targets, including native ants. In some cases such negative consequences may be avoided because invasive ants are behaviorally dominant and recruit to baits quicker preventing native ants from becoming exposed to the baits (Buczkowski and Bennett 2008). In other situations, however, native ants and other non-target invertebrates may succumb to the toxic effects of baits. The advantage of using live termites is that few native ants actively hunt termites. In contrast *P. chinensis* actively hunts termites (Matsuura 2002) and termites are its preferred prey (Bednar and Silverman 2011). Further, the use of subterranean termites (*Reticultermes* spp.) by *P. chinensis* has been shown to be a critical factor for their successful establishment in new habitats (Bednar and Silverman 2011). *P. chinensis* has never been shown to occur in habitats lacking subterranean termites (Rhinotermitidae) and the ants typically nest within decaying logs which are occupied by termites and ants. Termites naturally avoid light and air and field observations indicate that termites scattered on the ground immediately migrate towards the safety of leaf litter and other objects on the ground, the exact places where *P. chinensis* are nesting. Such termite behaviors effectively cause the termites to become "walking bait stations" whereby the termites deliver the insecticide directly to the ant colonies. This effectively eliminates the need for foraging and bait retrieval by the ants. Furthermore, *P. chinensis* disrupts ant-seed dispersal mutualisms by displacing native ant species, especially the keystone mutualist *Aphaenogaster rudis* (Rodriguez-Cabal et al. 2012). While both *A. rudis* and *P. chinensis* prey on termites (Buczkowski and Bennett 2007; Bednar and Silverman 2011), *P. chinensis* is able better utilize termite prey which may contribute to the displacement of *A. rudis* by *P. chinensis* (Bednar et al. 2013). Consequently, the use of poisoned termite prey in areas where the two species co-occur might lead to greater target selectivity and reduced non-target effects by selectively targeting *P. chinensis* and sparing *A. rudis*.

The baiting approach evaluated in this study may also provide significant environmental benefits with regard to pesticide residues in ecologically sensitive environments such as nature reserves, wilderness areas, national parks, and other protected landscapes where invasive ants pose a threat to native organisms and must be carefully managed to avoid non-target effects (e.g. Allen et al. 2004; Gerlach 2004; Plentovich et al. 2009). Traditional approaches to manage invasive ants include granular and liquid baits and such products typically contain relatively high concentrations of the active ingredient. In addition, commercial bait products are often applied at relatively high rates resulting in environmental persistence and non-target effects. The baiting approach evaluated in this study has the potential to significantly reduce the amount of the active ingredient, while providing a high level of efficacy. A study by Saran and Rust (2007) demonstrated that *Reticulitermes flavipes* termites exposed to sand treated with 25 ppm fipronil pick up approximately 10 ng fipronil. In the current study, 15 termites were utilized per 1 m^2, equivalent to approximately 150 ng fipronil per square meter. In contrast, a typical granular bait for ant control contains 0.015 % fipronil and is applied at the rate of 1 kg per 100 m^2, equivalent to 1.4×10^8 ng fipronil per square meter. This is over a million-fold higher than fipronil delivery via treated termites.

Future studies should focus on evaluating the Trojan horse baiting approach developed in this study for the control of other invasive organisms. While the idea of using poisoned prey has not been previously proposed or evaluated for managing invasive invertebrates, a similar approach has been tested for eradicating invasive brown treesnakes (*Boiga irregularis*) in Guam (Clark et al. 2012). Dead neonatal mice (*Mus musculus*) adulterated with acetaminophen were placed inside bait boxes where they were subsequently retrieved by the snakes. The poison baiting approach based on live termites (or other suitable prey) should also be tested against other invasive or pest ants, especially other specialist species that have relatively narrow feeding preferences and rely mostly on insect prey. The poison baiting approach might also be effective where leaf cutting ants are a pest in agricultural and natural areas. Vegetative matter treated topically with a toxicant might be effective in eradicating their large, subterranean colonies. The approach should also be tested with other insecticides, especially those with relatively low environmental persistence and low mammalian toxicity. Such approaches could especially be useful in environmentally sensitive areas where invasive ants threaten wildlife (e.g. ground-nesting sea birds) and secondary effects must be minimized. Finally, the poison baiting approach should be tested on a larger scale to better understand its efficacy at greater temporal and spatial scales and to determine its utility for area-wide eradications.

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