Research Article

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Fipronil-infused sodium polyacrylate gels provide effective management of Argentine ants in conservation areas

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Abstract

BACKGROUND: Various types of hydrogel compounds have recently been developed for controlling invasive and pest ants in a range of environmental settings including agricultural, urban and natural areas. The current study evaluated the potential of sodium polyacrylate (ACR) hydrogels to effectively deliver liquid baits to Argentine ants.

RESULTS: Relative to standard polyacrylamide (PAM) hydrogels, individual ACR hydrogel particles were approximately five-fold heavier; this may affect how ants interact with the bait particles, and further influence bait uptake and efficacy. Additionally, ACR hydrogels had significantly higher water absorption capacity and significantly slower rate of water loss, especially during the first 2 h. The efficacy of ACR hydrogel bait containing 0.005% fipronil and various attractants was evaluated on laboratory colonies. Results demonstrated that ACR hydrogel acceptance is significantly increased by the addition of feeding attractants. In addition, a field trial was performed in a nature reserve invaded by Argentine ants to evaluate the efficacy of ACR hydrogel bait. The field trial demonstrated that ACR hydrogel bait containing 0.005% fipronil with various attractants is highly effective and that ant densities throughout the baited plots declined by >99% within 7 days.

CONCLUSIONS: The results of this study demonstrate that: (i) fipronil is highly effective for Argentine ant control in natural areas when used in low concentrations (0.005%); (ii) ACR hydrogels are an effective tool for delivering liquid baits to Argentine ants; and (iii) hydrogel baits augmented with various attractants including salt, protein and pheromone are highly attractive to Argentine ants.

Keywords: Argentine ant; bait; hydrogels; fipronil; *Linepithema humile*; sodium polyacrylate; water-storing crystals

1 INTRODUCTION

The Argentine ant, *Linepithema humile*, is an invasive species that has successfully spread around the world and is a significant pest in urban, natural and agricultural habitats.[1–4] Colonies attain high numbers and form expansive supercolonies that threaten native ecosystems in North America, Europe, Hawaii, Australia and South Africa.[1,5] In South Africa, Argentine ants have invaded the Cape Floral Region, one of the world's most iconic centers of plant biodiversity.[6,7] This protected area, together with its highly biodiverse and endemic fynbos ecoregion, is one of the world's biodiversity 'hotspots'.[8] It also is the world's most endangered ecosystem as a consequence of a number of stressors including urban expansion, frequent fires, climate change and invasive species such as Argentine ants.[9] Argentine ants displace important seed-dispersing ant species[10] and deter pollinators from native plants that depend on insect pollination.[7,11]

Previous studies have evaluated several active ingredients and management approaches for broad-scale control of Argentine ant populations in natural ecosystems such as nature reserves and wilderness areas, but various regulatory and economic issues have limited the development of effective management tools. Current options for controlling Argentine ants in natural settings are limited to the application of residual insecticides and granular baits which are detrimental to the environment, labor-intensive to apply, and potentially disruptive to biological control.[4,12,13] Despite the tremendous economic and ecological impact of Argentine ants, effective management still faces many challenges, and control failures with liquid spray insecticides are common and well-documented in urban and natural areas.[4,14,15]

Recent advances in large-scale approaches to managing populations of Argentine ants in natural and agricultural systems include prey-baiting based on the use of insecticide-treated prey,[16,17] pheromone-assisted baiting,[18,19] trap–treat–release (TTR) based on horizontal insecticide transfer[20] and hydrogel baits.[21–24] Hydrogel baits have proven particularly effective;

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multiple studies have demonstrated they are highly efficacious in controlling Argentine ants in a wide range of urban, natural and agricultural environments across the world including Argentina,[23] Australia,[25] Japan,[26] South Africa[21] and the USA.[22,27]

Hydrogels are natural or synthetic 3D cross-linked polymer networks which can absorb and retain large amounts of water.[28] They are jelly-like solids with unique physical and biological properties, and have many applications in various processes ranging from industrial to biological.[29] More recently, hydrogels have proven highly effective in delivering liquid bait to invasive ants.[30] Among hydrogels, polyacrylamide copolymer (PAM) hydrogels and sodium polyacrylate hydrogels (ACR, known as waterlock) are the most common. A major difference between PAM and ACR is that the polyacrylamide has an amide group (NH_2) whereas the polyacrylate has a sodium (Na^+) group. Relative to PAM, ACR hydrogels are considered superabsorbent polymers and are substantially more absorptive. To date, all studies on the use of hydrogels for ant management have been performed with synthetic PAM hydrogels[13,22–26,30] or natural alginate hydrogels.[27,31] However, no study has evaluated the potential of ACR hydrogels in invasive ant management.

The current study had three main objectives. The first was to perform laboratory tests to compare various aspects of PAM *versus* ACR hydrogels including average particle size, water absorption capacity and water retention capability under outdoor conditions. The second objective was to perform a laboratory trial to evaluate the attractiveness and efficacy of PAM and ACR hydrogel baits containing 0.005% fipronil against colonies of Argentine ants. The prediction was that ACR hydrogels would be more effective in absorbing and retaining water, and would therefore be more attractive to and more effective against the target pest. The third objective was to perform a field study in a nature reserve to assess the toxicity of ACR hydrogel bait containing 0.005% fipronil and augmented with various attractants including salt, protein and pheromone against Argentine ants.

2 MATERIALS AND METHODS

2.1 Laboratory study to compare various aspects of PAM *versus* ACR hydrogels

A laboratory study was performed to compare various aspects of PAM *versus* ACR hydrogels including: (i) mean saturated particle weight, (ii) water absorption capacity and (iii) water loss under outdoor conditions. Mean saturated particle weight was estimated by weighting 20 randomly selected PAM or ACR hydrogel particles saturated in 25% sucrose solution. Mean particle weight (and related particle size) are important for granular bait retrieval and efficacy,[32] and previous work demonstrated that Argentine ants prefer particle sizes in the 840–1000 μm range.[32] The number of particles removed increased as particle size decreased, and ants generally preferred smaller particle sizes.[32] Additionally, a previous study revealed that Argentine ants imbibe liquid from PAM hydrogel, but do not carry individual hydrogel particles back to the nest as they are too heavy for individual ants to pick up.[23] Water absorption capacity was assessed by comparing the volume (milliliters) of 25% sucrose solution absorbed by 1.0 g dry PAM or ACR crystals ($n = 10$ for each hydrogel type). To assess water loss under outdoor conditions, 5 g PAM or ACR hydrogels saturated in 25% sucrose solution were placed in plastic weighing dishes and placed outdoors in a sunny location ($n = 6$ for each hydrogel type). The hydrogels were weighed at 1, 2, 3, 4, 6, 8, 10, 12, 24 and 48 h. The mean air temperature during outdoor exposure was 26 °C (min = 21 °C, max = 32 °C) and mean relative humidity (RH) was 73% (min = 54%, max = 92%).

2.2 Laboratory study on attractiveness and efficacy of PAM *versus* ACR hydrogels

The objective for the laboratory trial was to evaluate the relative attractiveness and efficacy of PAM and ACR hydrogels containing 0.005% fipronil, and augmented with various attractants against colonies of Argentine ants, *Linepithema humile*. The ants were collected from a large supercolony in Raleigh, North Carolina, and maintained on 25% sucrose solution and freshly-killed German cockroaches. Colonies were maintained and all experiments conducted at 27 ± 2 °C, 50 ± 50% RH, and 14 h:10 h light: dark cycle. To prepare 1 L hydrogel bait, 500 mL water was placed in a measuring container and 250 g sucrose was added. When the sugar dissolved, water was added to bring it up to 1 L to achieve 25% g mL^{-1} sucrose solution. Next, 0.5 mL Termidor SC (9.1% fipronil; BASF Corporation, Research Triangle Park, NC, USA) was added to 1 L sucrose solution to achieve 0.005% fipronil in 25% sugar water. The 0.005% concentration was based on fipronil concentration in commercially available ant baits which range from 0.01% to 0.001%, and a preliminary laboratory trial which confirmed the efficacy of 0.005% fipronil against *L. humile*. Next, three attractants were added to the liquid bait to make the bait more attractive to *L. humile*. Previous laboratory and field trials on the use of hydrogel baits for invasive ant management have only evaluated sucrose as an attractant. The attractants included salt, protein and pheromone. Salt was 1% sodium chloride (CAS no. 7647-14-5; Sigma Aldrich, St Louis, MO, USA) at 10 g NaCl in 1 L bait. A previous study demonstrated that ants have high affinity for sodium chloride and ants that have low access to salt in their diet prefer salt over sugar.[33] Protein was 1% sodium caseinate (CAS no. 9005-46-3; Sigma Aldrich) at 10 g sodium caseinate in 1 L bait. A previous study indicated that various proteins including sodium caseinate are highly attractive to *L. humile* and nutritionally complete for proper colony development.[34] Pheromone was (Z)-9-hexadecenal (CAS no. 56219-04-6; Bedoukian Research Inc., Danbury, CT, USA). Previous studies showed that foraging activity and mortality in *L. humile* are significantly improved by incorporating (Z)-9-hexadecenal into commercial baits.[18,19,26,35] The final pheromone concentration was 0.1 mg pheromone in 1 kg bait as used previously.[19] The objective was to evaluate the combined effect of the three attractants, not the relative attractiveness of each attractant. Once all attractants were incorporated into the liquid bait, the hydrogel, sodium polyacrylate (CAS no. 27599-56-0; Sigma Aldrich) was added to the mixture to absorb the liquid. Preliminary tests showed that the hydrogels reached maximum size and optimal saturation when 2.0 g dry hydrogel was mixed with 1 L bait. Hence, a ratio of 1 L of 0.005% fipronil in 25% sugar water to 2 g hydrogel was employed for bait preparation and the hydrogels were allowed ≥1 h to fully saturate.

The attractiveness and efficacy of three types of hydrogel baits containing 0.005% fipronil was evaluated: (1) standard PAM hydrogel, (2) ACR hydrogel without attractants, and (3) ACR hydrogel with the three attractants ($n = 5$ for each hydrogel type). Control tests ($n = 5$) consisted of colonies provisioned with 2 g ACR hydrogel saturated in sugar water containing no insecticide. Each hydrogel type was tested on colony fragments consisting of 500 workers, five queens and 1 g assorted brood. The colony fragments were placed inside 17 × 12 × 9 cm high Fluon-coated plastic boxes and allowed to colonize a moist plaster nest (5 cm diameter). The ants were provided with drinking water (ddH_2O) and allowed to acclimate to the nest for 48 h. No food was provided during the acclimation period. At the end of the acclimation period, 2 g hydrogel bait was introduced and the assay was run for 6 days. The attractiveness of hydrogel baits was estimated by monitoring worker recruitment at 15, 30 and 60 min for the first hour, and then every 30 min for 4 h (240 min total). For mortality, observations were made daily for 6 days (10:00 h) and consisted of (i) worker mortality, (ii) queen mortality and (ii) brood condition. Brood condition was recorded using a visual assessment and rated according to the following scale: 5 = no change from the original or more brood present (equivalent to 0% mortality), 4 = 70–90% brood present (15% mortality), 3 = 50% brood present (50% mortality), 2 = few brood present (90% mortality) and 1 = no brood present (100% mortality).

2.3 Field trial

Field plots containing colonies of *L. humile* were established at Helderberg Nature Reserve, Somerset West, Western Cape, South Africa (−34.06 S, 018.87 E). The plots were 10 × 10 m and were separated by ≥25-m buffer zones. To estimate initial ant densities [Day (D)0] the plots were sampled using note cards baited with a blend of canned tuna and corn syrup.[36] Within each plot, the bait cards were placed along two transects, 10-m-long perpendicular lines forming a cross through the center of each plot. Six evenly spaced cards were used along each transect (12 baits per plot). The cards were placed on the ground and collected 1 h after placement to estimate the number of Argentine ants present. Following census baiting, each 100 m^2 plot was baited with 600 g bait. The bait was prepared as above and contained 0.005% fipronil and the three attractants including NaCl, protein (sodium caseinate) and pheromone [(Z)-9-hexadecenal]. The bait was placed in roughly the same locations as the bait cards, equivalent to 12 50-g placements per plot. Bait efficacy was examined on D1, D3, D7, D14 and D21 using baited note cards as above. Six experimental plots and four control plots were established. Laboratory experiments indicated that the hydrogel bait lost a significant amount of water in the first 4 h. Therefore, bait applications were made at 08:00 h to minimize sun exposure and allow maximum foraging by Argentine ants. All assessments were performed from February to March 2024.

2.4 Statistical analyses

Student's *t*-tests were used to analyze the mean values of average particle size, water absorption capacity and water retention capability in PAM *versus* ACR hydrogels, and the mean number of ants attracted to ACR hydrogels with and without attractants. For all other laboratory trials and the field trial, a one-way repeated measures multivariate ANOVA test was used to examine the effect of treatment (i.e. hydrogel bait *versus* control), time and the interaction on ant number (for bait attractiveness trial) or ant percentage mortality counts for laboratory and field tests. Percentage mortality values were converted to a proportion and arcsine-transformed before the analysis. This was followed by univariate ANOVA to examine variation at each time point. Comparisons among treatments or among treatments over time consisted of ANOVA tests on mean cumulative percentage mortality followed by Tukey's honestly significant difference (HSD) method to test for significant differences among treatment means on each date. All statistical analyses were performed using Statistica 12.6.[37] The level of significance was set at $\alpha = 0.05$.

3 RESULTS

3.1 Laboratory study to compare various aspects of PAM *versus* ACR hydrogels

Results demonstrate that individual PAM hydrogel crystals are significantly heavier relative to ACR crystals. The mean particle weight for PAM hydrogels was 0.24 ± 0.06 g *versus* 0.05 ± 0.01 g for ACR hydrogels ($t = 13.6$, df = 28, $P < 0.001$). Therefore, individual PAM crystals are approximately five-fold heavier than individual ACR crystals which may affect how ants interact with the bait particles, and further influence bait uptake and efficacy. ACR hydrogels had significantly higher water absorption capacity relative to PAM hydrogels. A single gram of ACR hydrogel absorbed 418 ± 11 mL water *versus* 62 ± 4 mL for PAM hydrogel ($t = -913.9$, df = 18, $P = 0.01$). Both hydrogel types lost most of their weight in water within the first 8 h of outdoor exposure (Fig. 1). The duration of outdoor exposure had a highly significant effect on the amount of moisture lost by both hydrogel types (ANOVA, df = 14, $F = 123.2$, $P < 0.0001$). During the first 2 h, the rate of water loss was somewhat higher for PAM hydrogels *versus* ACR hydrogels. The difference was especially pronounced at 0.5 h, with water loss at 5 ± 2% for ACR *versus* 13 ± 2% for PAM (ANOVA, df = 5, $F = 4.3$, $P = 0.157$). Overall, however, the rate of water loss across the entire 48-h period was not statistically different between PAM and ACR hydrogels (ANOVA, df = 1, $F = 1.37$, $P = 0.473$). Little additional water loss was observed after the first 8 h. At 48 h, the percentage of water lost by ACR hydrogels was 98 ± 1% *versus* 99 ± 0% for PAM hydrogels (ANOVA, df = 5, $F = 0.54$, $P = 0.735$).

3.2 Laboratory study on attractiveness and efficacy of PAM *versus* ACR hydrogels

All hydrogel types were highly attractive to *L. humile*. Standard PAM hydrogel attracted a mean total of 150 ± 46 workers, ACR hydrogel 139 ± 43 workers and ACR hydrogel augmented with attractants 194 ± 40 workers over the 4 h testing period. Results demonstrate that ACR hydrogel acceptance is significantly increased by the addition of the three attractants ($t = -1.6$, df = 8, $P = 0.03$) (Table 1). However, PAM hydrogel was not significantly more attractive than ACR hydrogel without attractants ($t = 0.4$, df = 8, $P = 0.89$). Mortality in laboratory colonies provisioned with PAM, ACR and augmented ACR hydrogels was relatively quick: all colonies died within 6 days of being provided with the baits (Table 2). Overall, the effect of treatment (fipronil hydrogels *versus* control) was highly significant (ANOVA, df = 3, $F = 124.28$, $P < 0.0001$) indicating that 0.005% fipronil is highly effective against *L. humile* when delivered via hydrogel baits.

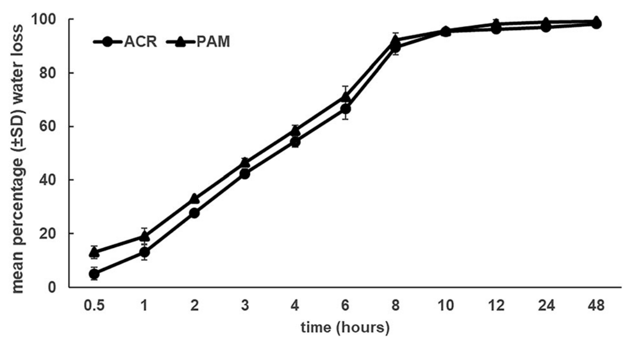

Figure 1. Mean percentage water loss (± SD) in PAM *versus* ACR hydrogels over a 48 h period.

Table 1. Mean total number (± SD) of *L. humile* workers recorded feeding on PAM and ACR hydrogel baits in laboratory assays

	Time (min)									
Hydrogel type	15	30	60	90	120	150	180	210	240	total
PAM	16 ± 7 b	30 ± 13 a	39 ± 19 a	22 ± 5 b	19 ± 10 b	8 ± 5 b	7 ± 4 a	6 ± 4 a	2 ± 2 a	150 ± 46 b
ACR	18 ± 8 b	29 ± 10 a	34 ± 10 a	24 ± 12 b	14 ± 13 b	8 ± 4 b	6 ± 4 a	3 ± 3 a	3 ± 3 a	139 ± 43 b
ACR + attractants	23 ± 9 a	34 ± 17 a	41 ± 10 a	32 ± 8 a	27 ± 7 a	20 ± 12 a	7 ± 4 a	4 ± 3 a	4 ± 3 a	194 ± 40 a

Totals within columns followed by the same letter are not significantly different ($P > 0.05$) based on Tukey's HSD test.

Table 2. Cumulative percentage mortality (± SD) in Argentine ant workers, queens and brood exposed to PAM and ACR hydrogels containing 0.005% fipronil

		Time (days)					
Hydrogel type	Caste	1	2	3	4	5	6
PAM	Workers	25 ± 9 a	85 ± 9 a	96 ± 4 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
ACR		31 ± 8 a	92 ± 7 a	99 ± 1 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
ACR + attractants		35 ± 7 a	90 ± 6 a	99 ± 2 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
Control		1 ± 1 b	1 ± 1 b	1 ± 1 b	2 ± 1 b	2 ± 1 b	2 ± 1 b
PAM	Queens	16 ± 17 a	24 ± 17 a	44 ± 22 a	68 ± 18 a	80 ± 14 a	100 ± 0 a
ACR		12 ± 18 a	56 ± 17 a	68 ± 18 a	84 ± 17 a	92 ± 11 a	100 ± 0 a
ACR + attractants		16 ± 17 a	32 ± 11 a	56 ± 17 a	72 ± 11 a	88 ± 11 a	100 ± 0 a
Control		0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
PAM	Brood	0 ± 0 a	19 ± 19 a	29 ± 19 a	82 ± 18 a	86 ± 21 a	100 ± 0 a
ACR		0 ± 0 a	26 ± 23 a	43 ± 16 a	66 ± 22 a	94 ± 5 a	100 ± 0 a
ACR + attractants		0 ± 0 a	19 ± 19 a	36 ± 19 a	74 ± 22 a	90 ± 0 a	100 ± 0 a
Control		0 ± 0 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b

Means within columns followed by the same letter within each caste are not significantly different by Tukey's HSD test ($P \leq 0.05$).

Results demonstrated a significant effect of day ($F = 75.3$, df = 7, $P < 0.0001$), and day × treatment interaction ($F = 18.4$, df = 21, $P < 0.0001$) with the majority of *L. humile* dying during the first 24 h. The effect of caste (workers *versus* queens *versus* brood) also was highly significant (ANOVA, df = 2, $F = 16.21$, $P = 0.002$) with workers dying significantly faster (Tukey's HSD test) than either queens or brood. The majority of workers (>85%) died within 2 days of bait introduction and complete worker mortality was achieved with all hydrogel types in 3 days (Table 2). Mortality in the queens was slightly slower during the first 2 days, but complete mortality was observed within 4–6 days. Mortality in the brood was somewhat delayed and a gradual deterioration of brood condition was observed. Within the brood, complete mortality was achieved by D6. Overall, however, the effect of hydrogel type was not significant and *L. humile* mortality was not significantly different among the three hydrogel types ($F = 97.2$, df = 16, $P = 0.12$). Mortality in the control treatment was ≤2%.

3.3 Field trial

Results of laboratory feeding trials and the field trial showed that ACR hydrogels augmented with attractants are highly attractive to *L. humile* (Fig. 2). Results from the field trial showed that *L. humile* can be effectively controlled using ACR hydrogel bait containing 0.05% fipronil and augments with attractants (Fig. 3). Within the treated plots, an average of 684 ± 341 ants per plot were detected during the initial pre-treatment inspection (Fig. 3). Relative to the initial counts, the ant densities throughout the baited plots declined significantly at D1 (108 ± 81 ants per plot; 84 ± 16% reduction; $t = 4.32$, df = 5, $P = 0.007$), D3 (49 ± 72 ants per plot; 92 ± 12% reduction; $t = 4.52$, df = 5, $P = 0.008$), D7 (7 ± 8 ants per plot; 99 ± 1% reduction; $t = 4.83$, df = 5, $P = 0.004$), D14 (62 ± 48 ants per plot; 88 ± 9% reduction; $t = 4.30$, df = 5, $P = 0.007$) and D21 (63 ± 59 ants per plot; 85 ± 17% reduction; $t = 3.94$, df = 5, $P = 0.001$) after treatment. Ant counts in the treated plots decreased sharply within 24 h of treatment and ≥99% reduction in ant counts was achieved within 7 days. Ant counts rebounded slightly on D14 and D21, but the mean level of control remained at ≥85% in the treated plots. Both treatment (ANOVA, df = 1, $F = 38.25$, $P < 0.0001$) and time (day) (ANOVA, df = 5, $F = 5.22$, $P < 0.0001$) were highly significant, whereas plot was not significant (ANOVA, df = 3, $F = 4.73$, $P = 0.42$). In control plots, an average of 771 ± 180 ants per plot were detected during the initial pre-treatment inspection, not significantly different from the 684 ± 341 ants detected in treatment plots (Student's *t*-test; $t = -1.05$, df = 3, $P = 0.37$). Ant counts in the control plots remained stable throughout the study and increased slightly on days 1 (+8%), 3 (+3%), 7 (+14%) and 14 (+37%). A substantial decrease in ant counts (−41%) was observed on D21 owing to adverse weather conditions.

4 DISCUSSION

One of the main goals for the current study was to assess the relative performance of polyacrylamide copolymer (PAM) *versus*

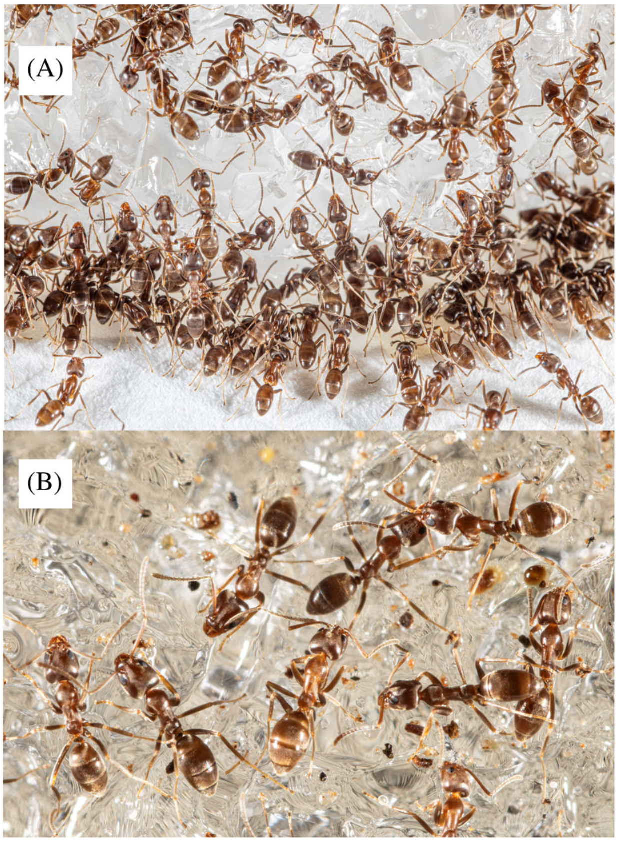

Figure 2. (A) Foraging Argentine ants workers feeding on ACR hydrogel bait; (B) close-up of Argentine ant workers feeding on ACR hydrogel with hydrogel crystal structure visible.

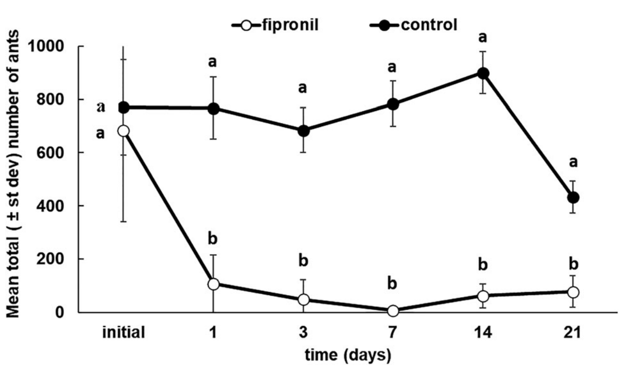

Figure 3. Mean total (± SD) number of *L. humile* workers detected on monitoring cards in plots treated with fipronil bait (open circles) and control plots (solid circles), at Day 0–21 after treatment. For each time point, the value represents an average of six treated plots or four control plots and 12 monitoring stations within each plot. Letters indicate pairwise differences in ant abundance at each assessment time between fipronil-treated and control plots.

sodium polyacrylate hydrogels (ACR). Relative to PAM, ACR hydrogels are considered true superabsorbent polymers and are substantially more absorptive. The prediction was that ACR hydrogels would be more effective in absorbing and retaining water and would therefore be more attractive and more effective against the target pest. To date, no study has evaluated the potential of ACR hydrogels in invasive ant management and all studies on the use of hydrogel baits for ant management have utilized PAM hydrogels[13,22–26,30] or natural alginate hydrogels.[27,31] Current results demonstrated that ACR hydrogels have a significantly higher water absorption capacity relative to PAM hydrogels. A single gram of dry ACR hydrogel absorbed approximately seven-fold more water than a gram of dry PAM hydrogel. Results also demonstrated that PAM hydrogels experienced a higher rate of water loss relative to ACR hydrogels. The rate of water loss for PAM hydrogels was especially higher during the first 2 h of outdoor exposure, a period that may be particularly important for bait uptake and bait efficacy. In the long-term, however, the amount of water lost by ACR and PAM hydrogels was >98% and not significantly different. Results of bait attractiveness tests demonstrated that ACR hydrogels are no more attractive than PAM hydrogels. However, ACR hydrogels augmented with attractants were significantly more attractive relative to ACR and PAM hydrogels without attractants. This suggests that the use of attractants in hydrogel baits may lead to faster bait discovery, increased feeding and bait intake, and potentially improvements in bait efficacy. Results also demonstrated that individual, fully-saturated PAM particles are approximately five-fold heavier than individual ACR particles. Bait particle weight (and related particle size) are important for granular bait retrieval and efficacy,[32] and previous work demonstrated that Argentine ants prefer particle sizes in the 840–1000 μm range.[32] The number of particles removed by Argentine ants increased as particle size decreased and the ants generally preferred smaller particle sizes.[32] The prediction for the current study was that *L. humile* would prefer ACR hydrogels over PAM hydrogels owing to smaller particle size and perhaps greater ease of handling. Despite these predictions, both hydrogel types appear equally attractive and results from the current laboratory study, corroborated by observations of feeding behavior in the field, demonstrate that *L. humile* do not carry individual ACR particles and simply feed directly by imbibing liquid sucrose solution from the hydrogels. A previous study evaluated the feeding behavior of *L. humile* on hydrogel baits and demonstrated that *L. humile* imbibe liquid from PAM hydrogel, but do not carry individual hydrogel particles back to the nest as they are too heavy for individual ants to pick up.[23]

Results of laboratory tests on the efficacy of PAM *versus* ACR hydrogels showed that both hydrogel types are highly attractive and equally effective against *L. humile*. Both hydrogel types required ~2 days to kill all workers, and 3–6 days to kill all queens and brood. No significant effect of hydrogel type was detected and *L. humile* mortality was not significantly different among the different hydrogel types. Additionally, ACR hydrogel bait augmented with attractants was as effective as standard ACR hydrogel, despite the augmented bait being substantially more attractive. This may be partially because fipronil is toxic to Argentine ants in extremely small amounts. A previous study examined the horizontal transfer of fipronil from treated *L. humile* workers to untreated workers and LC/MS/MS analysis demonstrated that 0.25 ng fipronil is sufficient to cause mortality in individual worker ants.[20] Therefore, fipronil is so effective against *L. humile* that the importance of formulation type and/or delivery method may be of minor importance relative to the active ingredient.

A secondary goal for the study was to evaluate a variety of feeding attractants that have previously been shown to be attractive to *L. humile* but have never been tested in combination with hydrogel baits. The attractants included NaCl, protein (sodium caseinate) and pheromone [(Z)-9-hexadecenal], and the objective was to evaluate their combined effect. Results of the laboratory feeding trial demonstrate that ACR hydrogel acceptance is significantly increased by the addition of the three attractants. Previous studies demonstrate that both NaCl[33] and sodium caseinate[34] are highly attractive to *L. humile* and necessary for proper colony development. Additionally, foraging activity and mortality in *L. humile* were significantly improved by incorporating *L. humile* trailing pheromone [(Z)-9-hexadecenal] into baits.[18,19,35] Although the addition of the attractants increased *L. humile* foraging activity in the laboratory, it is unclear if the same occurred in the field. Observations demonstrate that ACR hydrogel bait augmented with the attractants was highly attractive to *L. humile* in the field and resulted in ≥99% reduction in ant counts within 7 days. Previous studies demonstrated that *L. humile* consistently consume more liquid sucrose solution[35] and gel bait[19] which had been treated with (Z)-9-hexadecenal. It is hypothesized that the presence of (Z)-9-hexadecenal in the bait is important not only for attracting ants to the bait, but also for stimulating consumption and the frequency of trophallaxis among workers, resulting in increased efficacy.[19]

The results from the field study corroborate the results of laboratory tests and demonstrate that ACR hydrogel bait containing 0.005% fipronil and enhanced with attractants is highly effective in suppressing *L. humile*. Ant counts within the treated plots declined rapidly within 24 h of hydrogel application and *L. humile* activity declined by an average of 84% within 24 h. Peak *L. humile* suppression was achieved at D7 after treatment, with an estimated 99% reduction in activity from baseline counts and a 112-fold lower activity in treated plots in comparison with control plots. The study demonstrated that fipronil is a highly effective bait active ingredient for use against *L. humile*. Previous studies revealed that fipronil is highly toxic to *L. humile* via oral[32] and contact[38] exposures. Additionally, a laboratory study demonstrated that fipronil is highly effective against Argentine ants when deployed via alginate hydrogel beads in low (0.001%) and ultra-low (0.0001%) concentrations.[39] Previous field studies demonstrate that bait and spray applications of fipronil are highly effective in suppressing *L. humile* populations in urban, natural and agricultural settings.[4,17,20,40–42] Despite previous successes with fipronil, it has been rarely used as an active ingredient in hydrogel baits in field trials. Previous studies have mostly evaluated thiamethoxam and demonstrated that it is highly effective against Argentine ants in a variety of environments including commercial fruit orchard,[21] a large-scale eradication program in an ecological reserve,[43] an ecologically sensitive natural area in California Channel Islands,[22] commercial citrus groves[27] and a community-wide management program in an urban setting.[26] In addition to thiamethoxam, a previous field trial demonstrated that 0.01% spinosad had strong suppressive activity against Argentine ants in citrus orchards and was statistically equivalent to 0.0001% thiamethoxam.[24] To date, fipronil incorporated into hydrogel baits has been only tested in a single field trial, a broad-scale application to control *L humile* on Norfolk Island.[25] Hydrogel bait containing 0.006% fipronil was deployed via aerial dispersal using drones in natural areas with high abundance of *L. humile*. Although the treatment resulted in effective suppression of *L. humile*, it also unexpectedly caused honey bee death from aerosols inadvertently produced during aerial dispersal.[25] The result was unexpected because previous studies on nontarget effects of hydrogel baits have demonstrated lack of attractancy and impacts on bees and other nontarget insects.[44,45] The study concluded that aerial dispersal of hydrogels has inherently far greater risk for nontarget species than ground-based dispersal. Interestingly, a feral honey bee colony was present in one of the treatment plots in the current study. The colony nested in a tree hollow ≈5 m off the ground. Hydrogel bait was applied at the base of the tree and foraging Argentine ants were seen trailing into the canopy of the tree. The health of the honey bee colony was monitored during the course of the trial and no adverse effects were observed despite close proximity to the treatment and ants carrying the bait. A previous study assessed the attraction of honey bees to ground placements of hydrogel baits in a commercial apiary, and demonstrated that hydrogels are generally safe to lepidopteran and hymenopteran pollinators such as butterflies and honey bees.[44] Results from the current study support the conclusion of previous studies which demonstrated that ground applications of hydrogel baits are unlikely to have negative effects on nontargets, especially bees.

Previous approaches to control *L. humile* at the current study site, Helderberg Nature Reserve, included prey-baiting[17] and the TTR technique.[20] Both approaches utilized 0.06% fipronil and were highly effective against *L. humile*. The prey-baiting approach utilized termite prey topically treated with 0.06% fipronil and scattered in experimental plots invaded by *L. humile*. Results demonstrated rapid control of *L. humile* with 97% reduction at D1 and 99% reduction at D21 after treatment. Similar results were achieved with the TTR technique whereby *L. humile* workers were collected in invaded plots, topically treated with 0.06% fipronil, and released back into the plots. The treated workers then dispersed throughout the colony and disseminated fipronil to untreated nestmates through various social interactions. Results showed that the release of fipronil-treated workers reduced *L. humile* abundance within treated plots by >91% within 1 day and >99% control was achieved in 14 days. Results of the current study, which utilized 0.005% fipronil in a bait formulation, are similar to other approaches, and all three approaches appear highly suitable for controlling *L. humile* in conservation areas where nontarget effects are a concern.

In summary, results demonstrate that fipronil is highly effective for *L. humile* control in natural areas when used at a low concentration (0.005%) and deployed via ACR hydrogels containing multiple attractants. In particular, the use of (Z)-9-hexadecenal in hydrogel baits may offer land managers and pest management professionals a new tool to achieve satisfactory outcome in *L. humile* control/eradication programs. The pheromone is currently available commercially as BioAmp® AA (Suterra USA, Bend, OR, USA) in a sprayable formulation for use as adjuvant with nonrepellent insecticide sprays. The product lures ants into insecticide-treated areas, exposing more ants to the insecticide and greatly enhancing the effect. Future *L. humile* eradication efforts utilizing hydrogel baits should consider the use of various attractants to help boost efficacy and help achieve better success against *L. humile* which are notoriously difficult to eradicate.[4,15]

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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