Research Article

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Development of an alginate hydrogel to deliver aqueous bait for pest ant management

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Abstract

BACKGROUND: Insecticide sprays used for ant control cause environmental contamination. Liquid bait is a safe and effective alternative, but it requires bait stations to dispense the toxicant. We developed a biodegradable hydrogel to deliver liquid bait obviating the need for bait stations.

RESULTS: Alginate hydrogel beads with preferred rigidity and maximum hydration in 25% sucrose solution were engineered by optimizing a crosslinking process. The moisture content of the substrate on which the beads were placed and the relative atmospheric humidity significantly influenced water loss dynamics of the hydrated hydrogel beads. Laboratory choice studies indicated that hydrated hydrogel beads had reduced palatability to foraging ants when they lost ≥50% water. An enzyme-linked immunosorbent assay (ELISA) indicated that the insecticide thiamethoxam added to sucrose solution was absorbed into the hydrogel beads. Hydrogel beads conditioned in sucrose solution with 1 mg L^{-1} thiamethoxam provided complete control of all castes of Argentine ant *Linepithema humile* (Mayr) colony by 14 days post treatment in the laboratory trial and provided a 79% reduction in ant activity after 8 weeks in the field trial.

CONCLUSION: Alginate hydrogel beads provided an effective delivery system for liquid baits laced with low concentrations of insecticide to control Argentine ants.

Keywords: Argentine ant; ant control; biodegradable hydrogel; *Linepithema humile*; liquid bait; thiamethoxam

1 INTRODUCTION

The Argentine ant, *Linepithema humile* (Mayr), is an invasive pest with a worldwide distribution.[1,2] It is one of the most damaging pest ant species in urban,[3] agricultural,[4–6] and natural environments.[7–12] In common with other tramp ant species such as *Monomorium pharaonis* (L.),[13,14] *L. humile* has a high reproductive rate, polygynous colony structure, exhibits unicoloniality, and can propagate via budding.[14] Furthermore, similar to *M. pharaonis*,[15] Argentine ant colonies can survive and grow rapidly from a single queen tended by a few workers.[16,17] These attributes might have collectively contributed to the success of the Argentine ant as an invasive species in introduced ranges.[16,18]

Argentine ants often establish extensive area-wide infestations in urban settings,[19] making them a serious nuisance pest. For example, in urban residential settings of California, the Argentine ant is the most common pest ant species treated by pest management professionals.[3,20] In agricultural settings, Argentine ants readily establish trophobiotic relationships with honeydew-producing hemipteran pests.[21,22] Hemipteran honeydew is an important nutrient source for Argentine ants,[23] and the presence of Argentine ants harvesting honeydew protects hemipteran pests from natural enemies.[21,22] Thus, effective Argentine ant management in agricultural environments is necessary to maximize the effectiveness of natural enemies attacking plant pests.[21,24–28]

Owing to several practical advantages such as easy application and relatively quick suppression of pest ant populations, contact insecticide sprays are a common option for the control of Argentine ants in urban and agricultural settings.[3,22] Sprays containing phenylpyrazole or pyrethroids are applied by pest management professionals to control Argentine ants in urban residential settings;[19,29] and organophosphate insecticides may be used for pest ant control in citrus orchards and grape vineyards of California.[22,30] Consequently, some of these commonly used insecticides are frequently detected in waterways.[31–35] Also, certain types of agricultural pesticides used for ant control significantly contribute to volatile organic compounds emissions, potentially impacting air quality.[36]

Because of concerns over environmental contamination and effects on non-target organisms from spray insecticides used for ant control, liquid baits have been investigated for delivering low

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concentrations of insecticides in a highly targeted manner.[37–41] Sucrose liquid baits formulated with slow-acting toxicants delivered at precise concentrations have been demonstrated to effectively control Argentine ants.[42] However, there are several factors that may prevent the liquid baits from being widely adopted for ant management. For example, liquid baiting requires bait stations to contain and dispense the toxicant,[43] but the installation and maintenance of bait stations (inspection, cleaning, refilling, etc.) can be cost prohibitive.[37,43] The installation and maintenance of many bait stations over a large area are often necessary to achieve an acceptable level of ant control in the field.[37] In addition, there are challenges associated with bait station design, in which the liquid bait is typically stored in a reservoir and slowly released into a dispenser. The evaporation of water from the liquid bait in the dispenser can increase concentrations of bait and its active ingredient, causing the bait in the dispenser to be less palatable to foraging ants, which negatively impacts baiting efficacy.[44] Furthermore, the sucrose liquid baits contained in the bait reservoir can ferment under warm conditions, consequently compromising the attractiveness of the bait.[40,44]

To overcome the limitations of conventional liquid baiting, a hydrogel matrix has been recently studied as a method to deliver liquid baits to ants without using bait stations.[43,44] A synthetic hydrogel composed of polyacrylamide has been tested to deliver sucrose liquid baits targeting Argentine ants.[8,43–45] The use of hydrogel matrices makes it possible to apply liquid baits directly on the ground where ants are foraging. The highly absorbent hydrogel matrices act as a controlled-release vehicle as they keep the liquid bait palatable for an extended period by retaining water.[43–45] Polyacrylamide hydrogel, however, slowly degrades into its monomer, acrylamide, upon exposure to a temperature of 35 °C.[46] Acrylamide is listed as toxic chemical by the World Health Organization[47] and the State of California[48] as it is a potential peripheral nerve toxin and carcinogen.[48–50]

The use of natural hydrogel compounds that readily biodegrade without generating potentially toxic monomers would eliminate some of the aforementioned safety concerns. Here, we engineered a biodegradable hydrogel bait made from alginate, a naturally occurring polysaccharide derived from brown seaweeds, as an alternative hydrogel matrix for delivering liquid baits to Argentine ants. Alginates are polysaccharides consisting of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers of varying proportions and sequences.[51] During the crosslinking process, calcium ions from the calcium chloride ($CaCl_2$) solution replace the sodium ions in sodium alginate (Na-Alg) solution,[52,53] forming a solid calcium alginate hydrogel with a three-dimensional network structure.[54] Alginate hydrogels have been used to deliver various compounds such as fertilizers,[55] contact pesticides,[52,53,56] and pharmaceuticals.[57] However, several new developments were necessary to ensure that the alginate hydrogel system can deliver liquid baits with phagostimulants and toxicants at their optimal concentrations for maximum efficacy. Using thiamethoxam as the insecticidal active ingredient, laboratory and field studies were conducted to determine the effectiveness of the alginate hydrogel matrix to absorb and deliver sucrose liquid bait to the ants.

2 MATERIALS AND METHODS

Experiment 1 was conducted to identify a method to engineer alginate hydrogel (hereafter referred to as 'alginate hydrogel bead') with optimal physical rigidity and liquid bait uptake. Using the alginate hydrogel beads produced with the methods identified from experiment 1, experiments 2–5 were conducted to determine the potential of the alginate hydrogel bead as a matrix to deliver liquid baits targeting Argentine ants. Experiment 2 was conducted to determine water loss characteristics of alginate hydrogel beads under conditions with varying moisture levels. Experiment 3 was conducted to assess bait acceptance to foraging Argentine ants as alginate hydrogel beads desiccated. Experiment 4 characterized the hydration of the alginate hydrogel beads when they were conditioned in 25% sucrose solution containing different concentrations of thiamethoxam. Experiment 5 tested if thiamethoxam migrates into the entire alginate hydrogel bead's sponge-like matrix upon conditioning in the 25% sucrose solution with thiamethoxam. Lastly, experiments 6 and 7 were conducted to determine the efficacy of the alginate hydrogel beads containing thiamethoxam (hereafter referred to as 'alginate hydrogel bait') to control Argentine ants under laboratory and field conditions.

2.1 Experiment 1: engineering alginate hydrogel beads

Calcium alginate hydrogel beads were made by crosslinking sodium alginate (Na-Alg) solution ionotropically with calcium ions. Twenty-seven different combinations of Na-Alg and calcium chloride ($CaCl_2$) solution concentrations, and varying crosslinking times were investigated (Table 1). Either 1, 1.5, or 2 g of medium-viscosity Na-Alg (Sigma Aldrich, St. Louis, MO, USA) were mixed in 100 ml of deionized water to obtain solutions of 10, 15, or 20 g L^{-1} of Na-Alg, respectively. The mixtures were gradually heated to 60 °C while stirring to achieve complete dissolution of Na-Alg. Once cooled to room temperature, the Na-Alg solution was added to either 5, 10, or 20 g L^{-1} $CaCl_2$ solution, the crosslinker, using a 5 ml syringe (BD Bioscience, San Jose, CA, USA). The Na-Alg solution was dispensed dropwise from the syringe through a 100-mm long piece of Tygon tubing (Vincon flexible PVC tubing, 9.5 mm ID, 12.7 mm OD, 1.7 mm wall thickness; Saint-Gobain Performance Plastics, Garden Grove, CA, USA) with the end covered with a piece of fine fabric (30 × 30 mm). This modification allowed the accumulation of relatively large amount ($\sim$ 0.15 ml) of Na-Alg solution at the end of the tubing before the drop was detached and fell into the crosslinker, maximizing the size of hydrogel beads formed. The crosslinker was continuously stirred with magnetic stirring bar during this process. The resulting hydrogel beads were subsequently filtered from the crosslinker after 5, 15, or 30 min, and briefly rinsed with deionized water to remove the crosslinker from the surface. After gently removing excess moisture with laboratory tissue (Kimberly-Clark Professional, Roswell, GA, USA), each hydrogel bead was weighed on an analytical scale (AE 240, Mettler-Toledo, Columbus, OH, USA). This was recorded as the 'initial' weight. Each bead was then submerged in 100 ml of the 25% (w/v) sucrose solution (without toxicant) and 'conditioned' for 24 h to permit absorption of the 25% sucrose solution which resulted in the complete hydration of the beads. A 25% sucrose solution was chosen as the concentration for the bait because it is highly preferred by Argentine ants.[58] Following the 24-h conditioning period, the fully hydrated beads were removed from the sucrose solution and weighed after removing excess moisture on the surface. This was recorded as the 'final' weight. Each treatment was replicated 10 times.

Univariate General Linear Model (GLM) was used to evaluate the hydrogel percent weight gain [(final weight − initial weight)/initial weight × 100 (%)] across the different preparation parameters (i.e.,

Table 1. The 27 different combinations of hydrogel bead preparation conditions tested

Concentrations of Na-Alg (g L^{-1})	Concentrations of $CaCl_2$ (g L^{-1})	Crosslinking time (min)
10	5	5
10	5	15
10	5	30
10	10	5
10	10	15
10	10	30
10	20	5
10	20	15
10	20	30
15	5	5
15	5	15
15	5	30
15	10	5
15	10	15
15	10	30
15	20	5
15	20	15
15	20	30
20	5	5
20	5	15
20	5	30
20	10	5
20	10	15
20	10	30
20	20	5
20	20	15
20	20	30

Na-Alg and $CaCl_2$ solution concentrations, and crosslinking time), as well as the full factorial interaction effects of different preparation parameters. Stepwise multiple linear regression analysis was used to determine the predictive power of the variables that could account for a significant proportion of the variance in the regression model of percent weight gain.[59]

2.2 Experiment 2: water loss of alginate hydrogel beads

Water loss dynamics of alginate hydrogel beads were studied in simulated moisture conditions. To simulate varying moisture conditions of the ground surface and atmosphere, six different combinations of substrate moisture content and relative humidity (% RH) levels were tested.

Alginate hydrogel beads conditioned in a 25% sucrose solution were weighed and placed on the surface of moistened or dry sand (40 g, play sand, The Quikrete International Inc., Atlanta, GA, USA) contained in uncovered Petri dishes (100 mm in diameter and 15 mm in height). The moistened sand was prepared by adding 0.1 g of water per gram of sand while stirring, providing 10% (w/w) moisture level. For the dry sand treatment, the sand was used without added water. The sand dishes with hydrogel beads were placed in desiccators (240 mm in diameter) (Ace Glass, Inc., Vineland, NJ, USA), containing either 500 g of silica gel (0% RH), a saturated $MgCl_2$ salt solution (32% RH), or a saturated NaCl salt solution (75% RH). The desiccators were kept in an incubator at 25.6 °C. Temperature and humidity levels inside the desiccators were continuously recorded using HOBO UX100 detectors (Onset Computer Corp., Bourne, MA, USA). The hydrogel beads were weighed at 2, 4, 6, 8, and 24 h after placement in the desiccators. Sand particles attached to the surface of the hydrogel beads were carefully removed prior to weighing. After 24 h, all of the hydrogel beads were placed in a desiccator with 0% RH. The hydrogel beads were weighed daily until there was no further weight reduction following several successive measurements, indicating all water had been lost. The weight difference between the initial hydrogel bead and the completely dehydrated hydrogel bead was considered to be the total amount of water initially absorbed by the hydrogel bead. The initial total amount of water in the hydrogel bead was used to determine the percent water loss at a given time point. Experiments were replicated 10 times. Because the percent water loss data were not normally distributed, the data were arcsine square-root transformed prior to analysis to satisfy normality assumptions.[60] One-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test at the 0.05 level of significance were used to compare the data at each time point.[59]

2.3 Experiment 3: choice feeding study with partially dehydrated hydrogel beads

To understand the relationship between the loss of water from the hydrogel beads and feeding preference of Argentine ants, their feeding responses to the hydrogel beads with four levels of desiccation (i.e., 0, 25, 50, and 75%) were studied.

Colonies of *L. humile* were collected from a citrus grove located at the University of California, Riverside, CA, USA. Ants were extracted from collected soil, leaf litter, and other debris by thinly spreading these nesting materials within a large wooden box. Moist plaster of Paris nests were positioned at the center of the box. As the nesting materials desiccated, the ant colony moved into the moist plaster nests. The ant colony was then transferred into plastic containers which were maintained in the laboratory. For the choice feeding study, the experimental ant colony was placed in a polyethylene container (330 × 190 × 100 mm) the inner sides of which were coated with a thin film of Teflon (polytetrafluoroethylene suspension; BioQuip, Rancho Dominguez, CA, USA) to prevent the ants from escaping. Each colony consisted of 300 workers, two queens, and 0.1 g of brood that were deprived of sucrose solution for 3 days.

To prepare the hydrogel beads with 25, 50, and 75% water loss, they were first conditioned in 25% sucrose solution for 24 h and subsequently subjected to a constant moisture condition (0–5% RH on moistened sand) for approximately 2.5, 6.3, and 14.5 h, respectively (based on results from experiment 2, see Fig. 1). Bead weights were monitored constantly to prepare hydrogel beads with exactly 25, 50, and 75% water loss. Freshly conditioned hydrogel beads without the desiccation process were classified as beads with 0% water loss. Four individual hydrogel beads with 0, 25, 50, and 75% water loss were simultaneously placed on the bottom of the ant colony box in a square position, each bead was separated by 20 mm (Fig. 2). The numbers of ants feeding on the hydrogel beads were recorded based on digital pictures taken at 5, 15, 30, 45, and 60 min after beads were introduced to ant colonies. The experiment was replicated five times using five different ant colonies. Because the count data were not normally distributed and exhibited heterogeneity of variance, the data were $\log_{10}$ (+1) transformed where a constant was added to each number to account for a large number of zeros in the dataset prior to analysis.[60] One-way ANOVA and Tukey's HSD test

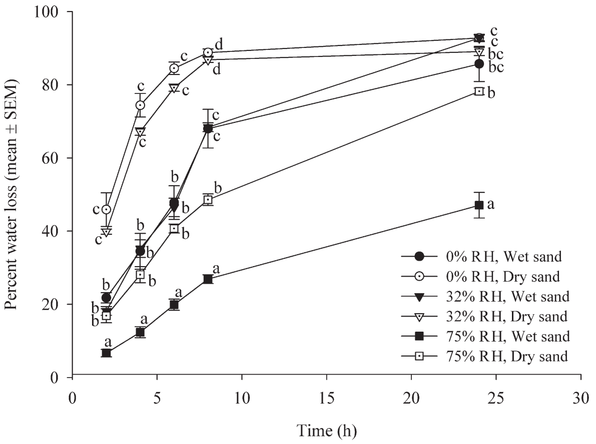

Figure 1. Percent water loss (mean ± SEM) of alginate hydrogel beads under various % RH and substrate moisture conditions. For each time point, values labeled with the same letters are not significantly different at $\alpha = 0.05$ (data were arcsine square-root transformed; Tukey's HSD).

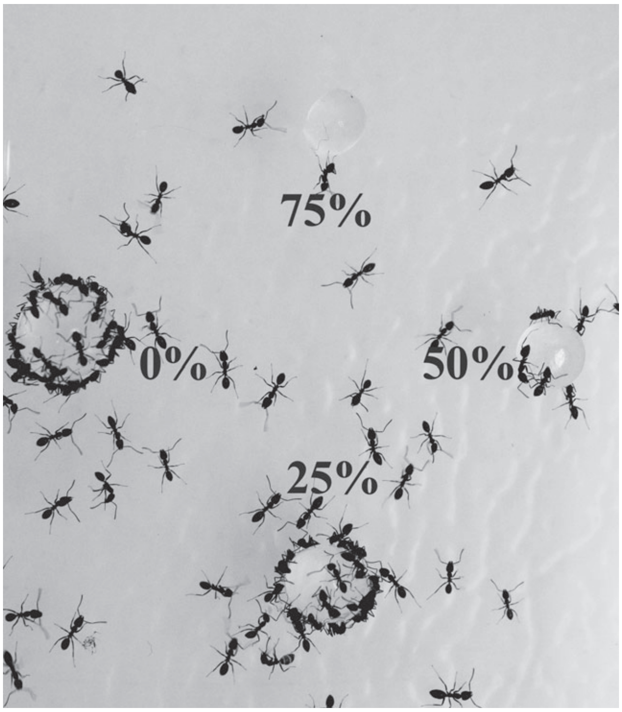

Figure 2. Choice feeding arena with hydrogel beads that lost 0, 25, 50, and 75% of water at 5 min post introduction into test arenas.

at the 0.05 level of significance were used to compare the data at each time point.[59]

2.4 Experiment 4: hydration of alginate hydrogel beads in sucrose solution containing thiamethoxam

Based on the results of experiment 1 (see below), 10 g L^{-1} Na-Alg solution, 5 g L^{-1} $CaCl_2$ solution, and a 5 min crosslinking time were used to produce alginate hydrogel beads for this experiment. Alginate hydrogel beads were briefly rinsed with deionized water, and the initial diameter of the bead was measured in mm using a Cen-tech digital caliper (Harbor Freight Tools, Camarillo, CA, USA). Bead weight (g) was measured with an analytical scale. Each bead was subsequently conditioned for 24 h in 100 ml of 25% sucrose solution with varying concentrations (0, 0.1, 0.4, 0.7, and 1 mg L^{-1}) of analytical grade thiamethoxam (Thiamethoxam PESTANAL®, Sigma Aldrich). As a negative control, hydrogel beads were conditioned in deionized water without sucrose and thiamethoxam. After the 24-h conditioning period, beads were removed from the solutions and excess moisture on the surface was gently removed using a laboratory tissue. The diameter and weight of the fully hydrated beads were measured. The experiment was replicated 10 times. One-way ANOVA and Tukey's HSD test at the 0.05 level of significance were used to compare the percent diameter increase and percent weight gain between the different treatments.[59]

2.5 Experiment 5: absorption of thiamethoxam into alginate hydrogel beads

To determine if thiamethoxam in the 25% sucrose solution was absorbed into the hydrogel matrix, the amounts of thiamethoxam in the outer and interior portions of the hydrogel bead were estimated using an enzyme-linked immunosorbent assay (ELISA). Details of this analytical method were described in Rust *et al.*[43] Each alginate hydrogel bead was conditioned in 100 ml of 25% sucrose solution containing 1 mg L^{-1} of thiamethoxam. Control hydrogel beads lacking thiamethoxam were conditioned in a 25% sucrose solution. After the 24-h conditioning period, hydrogel beads were removed from the solutions. To obtain hydrogel samples from the surface and from the inside of the beads to be analyzed by ELISA, the hydrogel beads were trimmed from the outside using a clean dissection knife leaving a small inner cube ($\sim 4 \times 4 \times 4$ mm). The final amount of the sample obtained from each part of hydrogel bead (i.e., small pieces from the outer portion or a small cube from the interior portion) weighed exactly 0.05 g. The samples were placed into separate 1.5-ml centrifuge tubes and 0.3 ml of distilled water was added to each tube. The hydrogel samples were homogenized with a plastic pestle and centrifuged (IEC Medilite microcentrifuge; Thermo Scientific, Waltham, MA, USA) for 5 min. Then, 4 μl of supernatant was pipetted out and diluted in 996 μl of distilled water (250-fold dilution). The amounts of thiamethoxam in the hydrogel samples were estimated using a commercially available ELISA kit (Thiamethoxam H.S. Plate Kit, catalog no. 20-0102, Beacon Analytical System Inc., Saco, ME, USA) as described by Byrne *et al.*[61] The experiment was replicated four times. The estimated amounts of thiamethoxam were compared between outer and interior portions of the hydrogel bead using a paired *t*-test at the 0.05 level of significance.[59]

2.6 Experiment 6: laboratory efficacy test

Efficacy of the alginate hydrogel baits containing thiamethoxam in 25% sucrose solution was tested with laboratory colonies of Argentine ants. Each experimental colony had 300 workers, two queens, and 0.1 g of brood (a mixture of eggs, larvae, and pupae) obtained from the main stock colony. The experimental ant colonies were kept in polyethylene containers (330 × 190 × 100 mm) the inner sides of which were coated with a thin film of Teflon. A Petri dish (100 mm in diameter and 15 mm in height) with four evenly distributed entry holes (4 mm in diameter) along the sides of the Petri dish, containing a piece of folded corrugated paper (140 × 60 mm), served as the artificial nest site. Once a week, ants were provided with water, a 25% sucrose solution, and fresh-killed cockroaches and canned tuna fish for protein. Ant colonies were acclimatized for 7 days before conducting experiments. Three days prior to the introduction of alginate baits containing sucrose solution with thiamethoxam, all food items except water were removed from colony boxes. Studies by Markin[62] showed that starvation of laboratory colonies for 3–4 days simulated the degree of foraging that would be seen in field populations of Argentine ant.

Efficacies of alginate hydrogel baits were tested with five different concentrations of thiamethoxam. The alginate hydrogel baits were conditioned in a 25% sucrose solution with 0.1, 0.4, 0.7, or 1 mg L^{-1} of analytical grade thiamethoxam. Three alginate hydrogel baits were placed on the bottom of the colony box. Control colonies were provided with alginate hydrogel baits conditioned in the sucrose solution only. At 24 h post treatment, the experimental colonies were returned to their regular diet. The hydrogel baits were not removed from the colony box.

Based on photographic images of the artificial nest sites and colony boxes, the number of live queens and workers were recorded at 1, 3, 5, 7, and 14 days post treatment. The weight of brood was also measured at these time points. The brood was typically found on the folded corrugated paper inside the artificial nest site. The weight of the brood was estimated by subtracting the weight of the folded corrugated paper from the weight of the folded corrugated paper with brood attached to it. The percent reductions in the number of workers and queens, and weight of brood were calculated. Each of the treatments and control was replicated five times. Because the percent reduction data were not normally distributed, the data were arcsine square-root transformed prior to analysis to satisfy normality assumptions.[60] Transformed data were analyzed using one-way ANOVA. Mean values were then separated with Tukey's HSD test at the 0.05 level of significance.[59]

2.7 Experiment 7: field efficacy test

The efficacy of the alginate hydrogel baits containing 1 mg L^{-1} of thiamethoxam (the most efficacious concentration tested based on the laboratory study; see Results) was tested at five residential houses in Riverside, CA, USA from July 28 to September 23, 2016. All sites had Argentine ant as the primary pest ant.

To increase the scale of production of alginate hydrogel baits for field experiments, droplets of 10 g L^{-1} Na-Alg solution was produced using a 100-nozzle shower head (AKDY AZ-6021 8-inch bathroom chrome shower head, AKDY Appliances, Rancho Cucamonga, CA, USA). The Na-Alg solution was slowly poured into a large funnel (150 mm in diameter) connected with the showerhead, and the droplets of Na-Alg solution from the showerhead were collected in a plastic container (381 × 292 × 152 mm) with 5 g L^{-1} $CaCl_2$ solution. The funnel plus showerhead was held by a clamp on a retort stand. The crosslinker was continuously stirred with a glass rod throughout this process to prevent the formed beads from adhering to each other. The resulting alginate hydrogel beads prepared from 5 L of Na-Alg solution were filtered out and conditioned in 5 L of 50% sucrose solution with 2 mg L^{-1} of thiamethoxam for 24 h. It was assumed that concentrations of thiamethoxam and sucrose solution inside and outside the hydrogel beads reached equilibrium by end of the 24-h conditioning period, which produced alginate hydrogel baits containing ~25% sucrose solution with ~1 mg L^{-1} of thiamethoxam. The hydrogel baits were sieved out from the liquid bait and stored in plastic jars at $4 \pm 1\,^{\circ}C$ until used.

Each experimental site was treated with ~1 kg of hydrogel baits at an application rate of 10 g m^{-2}. The hydrogel baits were applied in ~20 piles, mostly on active ant trails within 5 m of the building. Each pile consisted of ~50 g of alginate hydrogel baits. Estimation of foraging activity levels of Argentine ants before and after treatment were based on the amount of sucrose solution consumed by ants over a 24-h period. On each monitoring date, a total of 20 monitoring tubes (15 ml Falcon plastic tubes, BD Bioscience), each containing 12 ml of 25% sucrose solution, were placed at 10 different points evenly distributed along the perimeter of each house. A set of two tubes was placed at each point with the open end propped up in the notch of two Lincoln Logs™ (K'Nex Industries Inc., Hatfield, PA, USA) and covered with a flower pot (155 mm in diameter and 115 mm in height) to protect the tubes from sprinkler irrigation, pets, precipitation, and sunlight. The amount of sucrose solution consumed by the ants was estimated by measuring the difference between the initial and finial weights of the tubes over 24 h, and subsequently corrected for evaporation. The correction for evaporation was based on the weight loss from another set of monitoring tubes placed at another site in Riverside, CA, USA for 24 h, without the ants' access. Based on laboratory studies of Reierson *et al.*,[63] Argentine ants consume on average 0.3 mg of sucrose solution per visit. Based on this assumption, the number of ant visits to each tube was estimated, and the mean value between two tubes was used for further analyses. Field sites were monitored on day 1 pre-treatment, and weeks 1, 2, and 4 post treatment. The second treatment with hydrogel baits was made immediately after the monitoring at week 4, and sites were further monitored at weeks 5, 6, and 8 post treatment (calculated from the date of the first treatment). The amount of alginate hydrogel baits deployed per site and the method of application for the second application were identical to the first application.

Based on the visual inspection of the monitoring tubes upon pick up, only Argentine ants were found to be foraging in the monitoring tubes throughout the experimental period. The numbers of ant visits to each tube were estimated and recorded at each site for all monitoring dates. Because the ant visit data were not normally distributed and exhibited heterogeneity of variance, the data were square-root transformed prior to analysis.[60] The data at each post-treatment monitoring date were compared with the pre-treatment level with paired *t*-tests at the 0.05 level of significance.[59]

3 RESULTS

3.1 Experiment 1: engineering alginate hydrogel beads

The effects of Na-Alg solution concentration ($F = 124.2$; df = 2, $P < 0.001$), $CaCl_2$ solution concentration ($F = 1612.1$; df = 2, $P < 0.001$), and crosslinking time ($F = 1058.6$; df = 2, $P < 0.001$) on hydrogel percent weight gain were statistically significant (Table 2). Furthermore, significant interactions were observed among those three factors ($F = 27.1$; df = 8, $P < 0.001$) (Table 2). Multiple linear regression analysis of the hydrogel percent weight gain with the effects of Na-Alg solution concentration, $CaCl_2$ solution concentration, and crosslinking time gave correlation coefficients of $r = 0.141$, -0.661, and -0.529, respectively, indicating that all of the effects were significant ($P < 0.001$). The multiple regression model that determined the linear relationship between experimental variables was $Y = 352.6 + 49.0\ X_1 - 150.7\ X_2 - 7.3\ X_3$ ($R^2 = 0.737$, $F = 248.9$, df = 3, 266, $P < 0.001$) where Y = hydrogel percent weight gain, X_1 = Na-Alg solution concentration, X_2 = $CaCl_2$ solution concentration, and X_3 = crosslinking time. This equation indicated that a one-unit increase in the concentration of $CaCl_2$ solution and crosslinking time would decrease the hydrogel weight gain by 150.7 and 7.3%, respectively. Thus,

Table 2. Univariate GLM results for hydrogel percent weight gain under different combinations of preparation conditions

Source of variance	df[a]	*F*	*P*
Na-Alg concentration	2	124.16	< 0.001*
$CaCl_2$ concentration	2	1612.10	< 0.001*
Crosslinking time	2	1058.61	< 0.001*
Na-Alg concentration × $CaCl_2$ concentration	4	14.75	< 0.001*
Na-Alg concentration × crosslinking time	4	22.53	< 0.001*
$CaCl_2$ concentration × crosslinking time	4	130.25	< 0.001*
Na-Alg concentration × $CaCl_2$ concentration × crosslinking time	8	27.08	< 0.001*
Error	243		
Corrected total	269		

[a] df, degrees of freedom; *F*, *F* statistic.
*Significant at $\alpha = 0.05$.

to produce alginate hydrogel beads with highest percent weight gain upon conditioning, the method with 5 g L^{-1} $CaCl_2$ solution with 5 min crosslinking time was chosen. On the other hand, a one-unit increase in the concentration of Na-Alg solution would increase the hydrogel weight gain by 49%. However, 10 g L^{-1} Na-Alg solution was chosen because beads produced with >10 g L^{-1} Na-Alg solution disintegrated upon conditioning.

3.2 Experiment 2: water loss of alginate hydrogel beads

Hydrogel beads conditioned in 25% sucrose solution were exposed to six different combinations of moisture conditions (dry or moistened substrate, and 0, 32, or 75% RH) for 24 h. For the initial 8 h, the percent water losses for hydrogel beads were similar between 0 and 32% RH conditions when they were exposed to the same substrate moisture condition ($P > 0.05$) (see Fig. 1 for multiple comparisons). Throughout the entire experimental period (24 h), the hydrogel beads kept on the moistened substrate at 75% RH consistently had the lowest percent water loss compared with all other treatments (2 h, $F = 47.2$, df = 5, 54, $P < 0.001$; 4 h, $F = 66.4$, df = 5, 54, $P < 0.001$; 6 h, $F = 103.6$, df = 5, 54, $P < 0.001$; 8 h, $F = 87.0$, df = 5, 54, $P < 0.001$; 24 h, $F = 31.2$, df = 5, 54, $P < 0.001$) (Fig. 1).

3.3 Experiment 3: choice feeding study with partially dehydrated hydrogel beads

In general, foraging Argentine ants started to feed on all of the hydrogel beads immediately after their introduction to the colonies (Fig. 2). However, during the first 30 min post introduction, significantly fewer foraging ants were found on the hydrogel beads with 50 or 75% water loss, compared to the hydrogel beads with 0 or 25% water loss (5 min, $F = 58.5$, df = 3, 16, $P < 0.001$; 15 min, $F = 16.2$, df = 3, 16, $P < 0.001$; 30 min, $F = 30.9$, df = 3, 16, $P < 0.001$) (see Fig. 3 for multiple comparisons). In the following observations at 45 and 60 min, the hydrogel beads with 0 or 25% water loss were most attractive to ants, followed by beads with 50% water loss. Hydrogel beads with 75% water loss were least attractive to the foraging ants (45 min, $F = 22.8$, df = 3, 16, $P < 0.001$; 60 min, $F = 67.0$, df = 3, 16, $P < 0.001$) (Fig. 3).

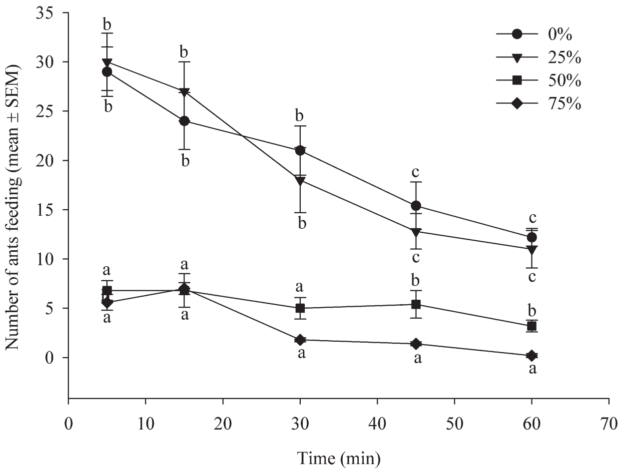

Figure 3. Number (mean ± SEM) of ants feeding on hydrogel beads over time. Legend shows different levels of water loss for the hydrogel beads upon testing. For each time point, symbols labeled with the same letters are not significantly different at $\alpha = 0.05$ [data were $\log_{10}(x + 1)$ transformed; Tukey's HSD].

3.4 Experiment 4: hydration of alginate hydrogel beads in sucrose solution containing thiamethoxam

The average initial diameter of the alginate hydrogel beads ranged from 5.81 to 6.00 mm across different solutions (Table 3). After conditioning, the average diameter of the hydrogel beads increased to 8.84–10.00 mm (Table 3). Percent increase in diameter was significantly larger for the beads conditioned in deionized water (67% increase in size) than for beads conditioned in the 25% sucrose solution (51–55% increase in size) ($F = 6.6$, df = 5, 54, $P < 0.001$). However, the hydrogel beads conditioned in 25% sucrose solutions (with or without thiamethoxam) had analogous percent increases in diameter ($P > 0.05$), suggesting the presence of thiamethoxam in the liquid bait at the tested concentrations (0.1–1 mg L^{-1}) did not influence the hydration of the hydrogel beads (Table 3).

The average initial weight of the alginate hydrogel beads was 0.14 g (Table 3). After conditioning, their average weights increased to 0.48–0.57 g (Table 3). Similar to the results with diameter, the beads conditioned in deionized water had significantly larger percent weight gain (309% increase) than the beads conditioned in the 25% sucrose solutions (253–272% increase) ($F = 7.2$, df = 5, 54, $P < 0.001$). The hydrogel beads conditioned in the sucrose solutions had similar percent weight gain regardless of the presence or absence of thiamethoxam ($P > 0.05$), further supporting the notion that thiamethoxam in the liquid bait did not influence the hydration of the hydrogel beads (Table 3).

3.5 Experiment 5: absorption of thiamethoxam into alginate hydrogel beads

The amounts of thiamethoxam per gram of hydrogel bead were similar between the outer and interior portions of the alginate hydrogel bead [1539.77 ± 93.05 and 1214.28 ± 50.69 ng (mean ± SEM) for outer and interior portions, respectively ($t = 2.3$, df = 3, $P > 0.05$)]. This indicated that thiamethoxam in the sucrose solution migrated evenly into the alginate hydrogel beads during the conditioning process. Although low level of absorbance was detected for the control hydrogel beads using an ELISA kit (Thiamethoxam H.S. Plate Kit), the absorbance values were negligible

Table 3. Percent diameter increase and percent weight gain of the hydrogel beads conditioned in various aqueous solutions

	Mean ± SEM					
Conditioning solution	Initial diameter (mm)	Final diameter (mm)	Diameter increase (%)[a]	Initial weight (g)	Final weight (g)	Weight gain (%)[a]
Deionized water	6.00 ± 0.05	10.00 ± 0.06	66.78 ± 1.75 a	0.14 ± 0.00	0.57 ± 0.01	309.19 ± 9.44 a
Blank bait solution (25% sucrose solution)	5.86 ± 0.10	8.91 ± 0.10	52.57 ± 2.92 b	0.14 ± 0.00	0.48 ± 0.00	254.57 ± 2.49 b
25% sucrose solution + 0.1 mg L^{-1} of thiamethoxam	5.81 ± 0.05	8.92 ± 0.07	53.69 ± 1.10 b	0.14 ± 0.00	0.49 ± 0.01	258.96 ± 4.82 b
25% sucrose solution + 0.4 mg L^{-1} of thiamethoxam	5.85 ± 0.05	8.84 ± 0.06	51.32 ± 1.37 b	0.14 ± 0.00	0.52 ± 0.01	271.64 ± 7.41 b
25% sucrose solution + 0.7 mg L^{-1} of thiamethoxam	5.91 ± 0.08	9.10 ± 0.09	54.23 ± 3.09 b	0.14 ± 0.00	0.49 ± 0.00	252.94 ± 6.03 b
25% sucrose solution + 1 mg L^{-1} of thiamethoxam	5.87 ± 0.05	9.08 ± 0.07	54.77 ± 2.11 b	0.14 ± 0.00	0.50 ± 0.01	266.26 ± 8.77 b

[a] Means followed by same letter within a column are not significantly different at $\alpha = 0.05$ (Tukey's HSD).

and potentially caused by a minor matrix effect (hydrogel and sucrose in the current study).

3.6 Experiment 6: laboratory efficacy test

Alginate hydrogel baits provided effective control of Argentine ant workers at tested concentrations of thiamethoxam (0.1–1 mg L^{-1}) (Table 4). No significant difference in the percent worker reduction was observed among all treated and untreated colonies at day 1 post treatment ($F = 1.7$, df = 4, 20, $P > 0.05$). However, at day 3 post treatment, significant differences in the percent worker reduction was recorded for colonies treated with the two higher concentrations (i.e., 0.7 and 1 mg L^{-1}) of thiamethoxam when compared with the control ($F = 39.9$, df = 4, 20, $P < 0.001$). Moreover, at day 5 post treatment, significant differences in the percent worker reduction were recorded for all treated colonies, when compared with the control ($F = 153.1$, df = 4, 20, $P < 0.001$). Colonies treated with the hydrogel baits conditioned in 1, 0.7, and 0.4 mg L^{-1} of thiamethoxam achieved 100% worker mortality by 5, 7, and 14 days post treatment, respectively (Table 4). About 21% of worker mortality was recorded in the control by day 14. However, the current control mortality was considered comparable with other similar laboratory studies in which 33% control mortality by day 8 post treatment[43] or 8–10% control mortality by day 7 post treatment[64] were recorded.

The hydrogel baits also provided effective control of queens and brood. At day 3 post treatment, the hydrogel baits conditioned in the solution with 1 mg L^{-1} of thiamethoxam provided significant reductions in queen number and brood quantity compared with the control (queen, $F = 16.0$, df = 4, 20, $P < 0.001$; brood, $F = 18.8$, df = 4, 20, $P < 0.001$). The hydrogel baits containing 0.4–1 mg L^{-1} of thiamethoxam provided 100% control for queens and brood by day 7 and 14 post treatment, respectively (Tables 5 and 6).

3.7 Experiment 7: field efficacy test

Average ant visits to monitoring tubes in all post treatment monitoring dates were significantly lower than their respective pre-treatment estimates throughout the entire experimental period (week 1, $t = 3.6$, df = 4, $P = 0.023$; week 2, $t = 3.6$, df = 4, $P = 0.022$; week 4, $t = 4.4$, df = 4, $P = 0.012$; week 5, $t = 5.7$, df = 4, $P = 0.005$; week 6, $t = 3.7$, df = 4, $P = 0.020$; week 8, $t = 6.9$, df = 4, $P = 0.002$) (Table 7). For the first two weeks post treatment, the hydrogel baiting provided widely variable control efficacies ranging from 7.8 to 65.1% reduction in ant visits. On average, 61–72% reductions in ant visits were recorded between weeks 4 and 6 post treatment with one site showing 88% reduction at week 4. By week 8 post treatment, ant visits were reduced by 64–91% when compared with the corresponding pre-treatment data. On average, this equated to an overall 79% reduction in ant visits.

4 DISCUSSION

The alginate hydrogel used in these experiments is a three-component system (alginate, water, and salts) in which each of the components can be modified to produce the hydrogel matrix with various properties (hardness, size, absorptivity, etc.).[65]

Table 4. Percent reduction of worker ants (mean ± SEM) in the laboratory study after baiting with alginate hydrogel baits

	Time (day)[a]				
Treatment	1	3	5	7	14
Control	9.87 ± 3.07a	12.13 ± 4.23a	13.93 ± 2.12a	11.80 ± 2.68a	21.20 ± 2.78a
0.1 mg L^{-1} of thiamethoxam	11.27 ± 2.33a	20.13 ± 6.94a	66.27 ± 3.83b	76.27 ± 3.25b	73.60 ± 3.04b
0.4 mg L^{-1} of thiamethoxam	15.67 ± 2.05a	22.07 ± 6.20a	57.60 ± 2.63b	85.73 ± 3.83b	100.00 ± 0.00c
0.7 mg L^{-1} of thiamethoxam	12.40 ± 5.01a	76.47 ± 3.47b	83.07 ± 3.39c	100.00 ± 0.00c	100.00 ± 0.00c
1 mg L^{-1} of thiamethoxam	22.93 ± 5.55a	89.07 ± 1.24b	100.00 ± 0.00d	100.00 ± 0.00c	100.00 ± 0.00c

[a] Means followed by same letter within a column are not significantly different at $\alpha = 0.05$ (data were arcsine square-root transformed; Tukey's HSD).

Table 5. Percent reduction of queen ants (mean ± SEM) in the laboratory study after baiting with alginate hydrogel baits

	Time (day)[a]				
Treatment	1	3	5	7	14
Control	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
0.1 mg L^{-1} of thiamethoxam	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	40.00 ± 18.71b	60.00 ± 18.71b
0.4 mg L^{-1} of thiamethoxam	0.00 ± 0.00a	0.00 ± 0.00a	30.00 ± 12.25ab	100.00 ± 0.00c	100.00 ± 0.00c
0.7 mg L^{-1} of thiamethoxam	0.00 ± 0.00a	0.00 ± 0.00a	50.00 ± 15.81b	100.00 ± 0.00c	100.00 ± 0.00c
1 mg L^{-1} of thiamethoxam	0.00 ± 0.00a	40.00 ± 10.00b	100.00 ± 0.00c	100.00 ± 0.00c	100.00 ± 0.00c

[a] Means followed by same letter within a column are not significantly different at $\alpha = 0.05$ (data were arcsine square-root transformed; Tukey's HSD).

The physical and chemical properties of the hydrogel matrix could affect the release of compounds incorporated in the hydrogel.[52] Thus, the versatility of the alginate hydrogel system would make it highly useful for developing novel baiting systems with various types of phagostimulants and insecticidal active ingredients. In this study, the high absorbency of the aqueous sugar bait with the active ingredient thiamethoxam was considered one of the optimal properties for the alginate hydrogel beads. The firm, spherical hydrogel beads would facilitate application of the hydrogel baits in the field either by motorized tools or by hand.[43]

Concentrations of Na-Alg solution, $CaCl_2$ solution, and crosslinking time significantly influenced the degree of hydration of alginate hydrogel beads in the 25% sucrose solution. First, the degree of hydration was positively correlated with Na-Alg solution concentration. Hydrogel produced from a polymer solution with higher concentrations would contain more polymers per unit amount of hydrogel,[52] potentially allowing it to absorb larger amounts of water. However, in the current study, some of the hydrogel beads produced from Na-Alg solutions at higher concentrations (15 and 20 g L^{-1}) experienced physical disintegration during the conditioning process due to over-hydration. Alginate hydrogels produced from 10 g L^{-1} Na-Alg solution maintained a firm spherical shape after the conditioning process. Second, the degree of hydration of the alginate hydrogel bead was negatively correlated with crosslinker concentration and crosslinking time. Higher concentrations of crosslinker and longer crosslinking times increased crosslink density in the hydrogel bead, consequently resulting in higher rigidity and decreased hydration potential.[52,54] The increase of crosslink density could also reduce the pore sizes in hydrogel matrix, restraining the uptake and release of water and other compounds dissolved in water.[53,66] To maximize the hydration potential, the current study used the lowest values for crosslinker concentration (5 g L^{-1}) and crosslinking time (5 min) to produce hydrogel beads.

Argentine ants in urban and agricultural settings show the highest foraging activity during the warm summer months, while preferring locations where moisture is available.[67] Consequently, alginate hydrogel baits targeting Argentine ants will be exposed to varying moisture conditions during field use. Because the moisture content in the hydrogel bait impacts palatability to foraging ants,[43] the water loss dynamics of the hydrated hydrogel beads needed evaluation. Alginate hydrogel beads lost water more quickly when they were exposed to dry substrates and/or low % RH. Interestingly, under low or intermediate atmospheric humidity conditions (i.e., 0 and 32% RH), water loss was mostly determined by moisture levels in the substrate rather than the atmospheric moisture level. It is possible that alginate hydrogel beads were capable of absorbing water from the moistened substrate, which compensated for water loss through surface evaporation. The water loss dynamics of alginate hydrogel beads (68–89% water loss during the first 8 h at 0–32% RH) were comparable with that of polyacrylamide hydrogels reported in Buczkowski *et al.*,[44] who reported that most of water was lost during the first 8 h upon exposure to outdoor conditions of 20–32 °C and 22–49% RH (70% weight loss was erroneously used as percent water loss in Buczkowski *et al.*[44]).

The choice feeding study indicated that alginate hydrogel beads with ≥50% water loss showed reduced palatability or attractiveness to foraging ants, similar to the findings of Rust *et al.*[43] for polyacrylamide hydrogel. Because the active ingredient, thiamethoxam, in the sucrose solution was not deterrent to foraging Argentine ants at the rates tested in the current study (0.1–1 mg L^{-1}, 10 fold difference), the moisture content of the hydrogel beads would be the most important factor for the continued foraging by the ants (J.W.T., unpublished data). For practical application in the field, it would be beneficial to minimize water loss from the hydrogel baits so that they remain palatable to foraging ants for longer periods. This could be achieved by applying hydrogel baits close to the irrigation points or increasing the moisture level of

Table 6. Percent reduction of brood ants (mean ± SEM) in the laboratory study after baiting with alginate hydrogel baits

	Time (day)[a]				
Treatment	1	3	5	7	14
Control	10.00 ± 6.32ab	−2.00 ± 12.41a	16.00 ± 8.72a	16.00 ± 5.10a	14.00 ± 7.48a
0.1 mg L^{-1} of thiamethoxam	6.00 ± 4.00ab	26.00 ± 6.78ab	20.00 ± 4.47a	36.00 ± 5.10a	52.00 ± 3.74b
0.4 mg L^{-1} of thiamethoxam	0.00 ± 0.00a	36.00 ± 5.10b	58.00 ± 3.74b	98.00 ± 2.00b	100.00 ± 0.00c
0.7 mg L^{-1} of thiamethoxam	36.00 ± 9.80c	74.00 ± 6.78c	76.00 ± 4.00b	94.00 ± 2.45b	100.00 ± 0.00c
1 mg L^{-1} of thiamethoxam	22.00 ± 5.83bc	78.00 ± 7.35c	100.00 ± 0.00c	100.00 ± 0.00b	100.00 ± 0.00c

[a] Means followed by same letter within a column are not significantly different at $\alpha = 0.05$ (data were arcsine square-root transformed; Tukey's HSD).

Table 7. Pre- and post treatment average ant visits (mean ± SEM) at five sites treated with hydrogel baits containing 1 mg L^{-1} of thiamethoxam

Site[a]	Pre-treatment average ant visits	Post-treatment average ant visits[b] (% reduction)[c] 1 week	2 weeks	4 weeks	5 weeks	6 weeks	8 weeks
1	54,199 ± 233	26,166 ± 162 (51.7)	32,707 ± 181 (39.7)	18,474 ± 136 (65.9)	15,058 ± 123 (72.2)	22,236 ± 149 (59.0)	13,261 ± 115 (75.5)
2	70,197 ± 265	24,508 ± 157 (65.1)	30,217 ± 174 (57.0)	8,284 ± 91 (88.2)	13,871 ± 118 (80.2)	9,048 ± 95 (87.1)	6,229 ± 79 (91.1)
3	30,517 ± 175	26,925 ± 164 (11.8)	28,133 ± 168 (7.8)	23,460 ± 153 (23.1)	18,316 ± 135 (40.0)	26,294 ± 162 (13.8)	11,033 ± 105 (63.8)
4	46,826 ± 216	32,490 ± 180 (30.6)	29,689 ± 172 (36.6)	8,029 ± 90 (82.9)	5,887 ± 77 (87.4)	15,180 ± 123 (67.6)	5,672 ± 75 (87.9)
5	68,741 ± 262	35,272 ± 188 (48.7)	30,304 ± 174 (55.9)	15,077 ± 123 (78.1)	14,253 ± 119 (79.3)	17,474 ± 132 (74.6)	15,314 ± 124 (77.7)
Average	54,096 ± 16,451	29,072 ± 4,583* (41.5 ± 20.7)	30,210 ± 1,645* (39.5 ± 19.9)	14,665 ± 6,648* (67.7 ± 26.2)	13,477 ± 4,590* (71.8 ± 18.6)	18,046 ± 6,616* (60.5 ± 28.0)	10,302 ± 4,255* (79.2 ± 10.8)

[a] Yards of five separate residential houses (Riverside, CA, USA).
[b] A second bait treatment was made at all sites between week 4 and 5.
[c] Each data point is the % reduction from corresponding pre-treatment average ant visits.
*Significant at $\alpha = 0.05$ compared with corresponding pre-treatment average ant visits (data were square-root transformed; paired *t*-test).

the substrate (i.e., soil) by irrigation before applying the hydrogel baits. Alternatively, the initial discovery and consumption of the bait by foraging ants could be enhanced before the hydrogels lose too much moisture. Boser *et al.*[8] observed that 66% of Argentine ants visit polyacrylamide hydrogel baits within 4 h after the initial application in the field. Argentine ant trail pheromone could be incorporated in the alginate hydrogel baits to reduce initial bait discovery time. This aspect warrants future study.

Thiamethoxam was chosen as the insecticidal active ingredient for baiting because of its relatively high water solubility of 4.1 g L^{-1} at 25 °C.[42] The high water solubility of the active ingredient is recognized as one of the important properties to formulate an efficacious aqueous bait.[42] The ELISA study revealed that thiamethoxam dissolved in the sucrose solution diffused through the alginate hydrogel matrix effectively. The results suggest that thiamethoxam will be continuously accessible as ants imbibe the sucrose solution from the surface of hydrogel beads. The concentrations of thiamethoxam were almost identical between the hydrogel matrix and the original liquid bait (i.e., 1.2–1.5 mg kg^{-1} vs. 1 mg L^{-1} for the hydrogel matrix and original liquid bait, respectively), suggesting that the liquid in freshly made hydrogel beads was effectively replaced by sucrose–thiamethoxam solution through diffusion, and equilibrium was probably achieved during the 24-h conditioning period.

The laboratory study indicated that alginate hydrogel baits with low concentrations of thiamethoxam (0.1–1 mg L^{-1}) were effective in controlling Argentine ant queens by day 7 post treatment. Because Argentine ant queens do not typically forage outside of the nest, the bait with toxicants are typically delivered to the queens via worker foraging and subsequent trophallaxis.[62] Thus, potential dilutions of the toxicants via trophallaxis and other means has been considered as one of the critical challenges in controlling reproductive queens with liquid baiting. In previous studies with alginate hydrogel to deliver chemicals, the chemicals were typically added in the alginate solution before the crosslinking process.[52,53,56] However, we recognized that the crosslinking process may inadvertently affect the concentrations of phagostimulant (sucrose) and insecticidal active ingredient (thiamethoxam) in the alginate hydrogel bait. Thus, we prepared the alginate hydrogel beads first and subsequently conditioned them in the 25% sucrose solution containing known concentrations of thiamethoxam. With this new approach, we were able to ensure that known amounts of sucrose and thiamethoxam were incorporated into the final (fully hydrated) baits to maximize their efficacy.

The concentration of 1 mg L^{-1} of thiamethoxam was chosen for the field study because it provided significant control in workers, queens, and brood by day 3 post treatment in the laboratory study. The alginate hydrogel baits immediately provided a 42% ant reduction on average by week 1 post treatment, and maintained 40–68% ant reduction by week 4. After a second treatment between weeks 4 and 5, the alginate hydrogel baiting maintained 61–79% ant reductions on average until the end of experiment. Based on weekly monitoring of an untreated field site, ant activity levels around the study area did not experience any natural decline throughout the entire field study period (J.W.T., unpublished data). Additionally, the average temperature range throughout the study was 20.6–32.2 °C, which is well within the temperature range which would allow normal foraging activity of Argentine ants.[68]

The use of alginate hydrogel matrix to deliver a sucrose liquid bait with low amounts of insecticide would reduce undesirable environmental impacts by eliminating the accumulation of synthetic hydrogel compounds (e.g., acrylamides) while allowing effective ant management. A total of 10 mg of thiamethoxam was used for a total of 10 kg of baits used for two field treatments. This is an extremely small amount (1 mg L^{-1} of thiamethoxam) compared with other commercial bait products containing thiamethoxam (e.g., Optigard Ant Gel Bait that contains ~100 mg L^{-1} of thiamethoxam). Although comparable with polyacrylamide hydrogels in terms of several properties (i.e., hydration, water loss rate, efficacy), the alginate hydrogel is environmentally friendly because it does not leave any potentially toxic monomers on degradation. Unlike other natural gel compounds (e.g., gelatin), the heat-stability of the alginate hydrogel[65] makes it suitable for use in areas where daily high temperatures exceed 35 °C. Alginates are also commercially available and relatively inexpensive, making this material attractive for large scale production. Future studies to determine the water loss and palatability dynamics of the alginate hydrogel baits in the field would help to design more effective baiting programs. Future research is also warranted to explore the utility of other active ingredients, in addition to thiamethoxam, for incorporation within the alginate hydrogel matrix. Additional field work is currently underway to assess alginate hydrogels for ant control in agriculture (e.g., citrus orchards), where pest ants present a perennial management problem.

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REFERENCES

1 Wild AL, Taxonomy and distribution of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). *Ann Entomol Soc Am* **97**:1204–1215 (2004).

2 Wetterer JK, Wild AL, Suarez AV, Roura-Pascual N and Espadaler X, Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). *Myrmecological News* **12**:187–194 (2009).

3 Knight RL and Rust MK, Repellency and efficacy of insecticides against foraging workers in laboratory colonies of Argentine ants (Hymenoptera: Formicidae). *J Econ Entomol* **83**:1402–1408 (1990).

4 Daane KM, Cooper ML, Sime KR, Nelson EH, Battany MC and Rust MK, Testing baits to control Argentine ants (Hymenoptera: Formicidae) in vineyards. *J Econ Entomol* **101**:699–709 (2008).

5 Daane KM, Sime KR, Hogg BN, Bianchi ML, Cooper ML, Rust MK *et al.*, Effects of liquid insecticide baits on Argentine ants in California's coastal vineyards. *Crop Prot* **25**:592–603 (2006).

6 Vega SJ and Rust MK, The Argentine ant – a significant invasive species in agricultural, urban and natural environments. *Sociobiology* **37**:3–25 (2001).

7 Bond W and Slingsby P, Collapse of an ant–plant mutalism: the Argentine ant (*Iridomyrmex humilis*) and *Myrmecochorous proteaceae*. *Ecology* **65**:1031–1037 (1984).

8 Boser CL, Hanna C, Faulkner KR, Cory C, Randall JM and Morrison SA, Argentine ant management in conservation areas: results of a pilot study. *Monogr West N Am Nat* **7**:518–530 (2014).

9 Holway DA, Lach L, Suarez AV, Tsutsui ND and Case TJ, The causes and consequences of ant invasions. *Annu Rev Ecol Syst* **33**:181–233 (2002).

10 Suarez AV, Richmond JQ and Case TJ, Prey selection in horned lizards following the invasion of Argentine ants in southern California. *Ecol Appl* **10**:711–725 (2000).

11 Choe DH, Vetter RS and Rust MK, Development of virtual bait stations to control Argentine ants (Hymenoptera: Formicidae) in environmentally sensitive habitats. *J Econ Entomol* **103**:1761–1769 (2010).

12 Hoffmann BD, Luque GM, Bellard C, Holmes ND and Donlan CJ, Improving invasive ant eradication as a conservation tool: a review. *Biol Conserv* **198**:37–49 (2016).

13 Tay JW and Lee CY, Induced disturbances cause *Monomorium pharaonis* (Hymenoptera: Formicidae) nest relocation. *J Econ Entomol* **108**:1237–1242 (2015).

14 Passera L, Characteristics of tramp species, in *Exotic Ants: Biology, Impact, and Control of Introduced Species*, ed. by Williams, Westview, Boulder, CO, pp. 23–43 (1994).

15 Tay JW, Neoh KB and Lee CY, The roles of the queen, brood, and worker castes in the colony growth dynamics of the pharaoh ant *Monomorium pharaonis* (Hymenoptera: Formicidae). *Myrmecol News* **20**:87–94 (2014).

16 Tsutsui ND and Suarez AV, The colony structure and population biology of invasive ants. *Conserv Biol* **17**:48–58 (2003).

17 Hee JJ, Holway DA, Suarez AV and Case TJ, Role of propagule size in the success of incipient colonies of the invasive Argentine ant. *Conserv Biol* **14**:559–563 (2000).

18 Silverman J and Brightwell RJ, The Argentine ant: challenges in managing an invasive unicolonial pest. *Annu Rev Entomol* **53**:231–252 (2008).

19 Klotz JH, Rust MK, Field HC, Greenberg L and Kupfer K, Controlling Argentine ants in residential settings (Hymenoptera: Formicidae). *Sociobiology* **51**:579–588 (2008).

20 Field HC, Evans WE, Hartley R, Hansen LD and Klotz JH, A survey of structural ant pests in the southwestern USA (Hymenoptera : Formicidae). *Sociobiology* **49**:151–164 (2007).

21 Tena A, Hoodle CD and Hoodle MS, Competition between honeydew producers in an ant–hemipteran interaction may enhance biological control of an invasive pest. *Bull Entomol Res* **103**:714–723 (2013).

22 Moreno DS, Haney PB and Luck RF, Chlorpyrifos and diazinon as barriers to Argentine ant (Hymenoptera, Formicidae) foraging on citrus trees. *J Econ Entomol* **80**:208–214 (1987).

23 Shik JZ and Silverman J, Towards a nutritional ecology of invasive establishment: aphid mutualists provide better fuel for incipient Argentine ant colonies than insect prey. *Biol Invasions* **15**:829–836 (2012).

24 Pekas A, Tena A, Aguilar A and Garcia-Marí F, Effect of Mediterranean ants (Hymenoptera: Formicidae) on California red scale (Hemiptera: Diaspididae) populations in citrus orchards. *Environ Entomol* **39**:827–834 (2010).

25 Juan-Blasco M, Tena A, Vanaclocha P, Cambra M, Urbaneja A and Monzó C, Efficacy of a micro-encapsulated formulation compared with a sticky barrier for excluding ants from citrus canopies. *J Appl Entomol* **135**:467–472 (2011).

26 Calabuig A, Garcia-Marí F and Pekas A, Ants affect the infestation levels but not the parasitism of honeydew and non-honeydew producing pests in citrus. *Bull Entomol Res* **104**:405–417 (2014).

27 Calabuig A, Tena A, Wäckers FL, Fernández-Arrojo L, Plou FJ, Garcia-Marí F *et al.*, Ants impact the energy reserves of natural enemies through the shared honeydew exploitation. *Ecol Entomol* **40**:687–695 (2015).

28 Calabuig A, Garcia-Marí F and Pekas A, Ants in citrus: impact on the abundance, species richness, diversity and community structure of predators and parasitoids. *Agric Ecosyst Environ* **213**:178–185 (2015).

29 Klotz JH, Rust MK, Greenberg L, Field HC and Kupfer K, An evaluation of several urban pest management strategies to control Argentine ants (Hymenoptera: Formicidae). *Sociobiology* **50**:391–398 (2007).

30 Tollerup KE, Rust MK, Dorschner KW, Phillips PA and Klotz JH, Low-toxicity baits control ants in citrus orchards and grape vineyards. *Calif Agric* **58**:213–217 (2004).

31 Delgado-Moreno L, Lin K, Veiga-Nascimento R and Gan J, Occurrence and toxicity of three classes of insecticides in water and sediment in two southern California coastal watersheds. *J Agric Food Chem* **59**:9448–9456 (2011).

32 Gan J, Bondarenko S, Oki L, Haver D and Li JX, Occurrence of fipronil and its biologically active derivatives in urban residential runoff. *Environ Sci Technol* **46**:1489–1495 (2012).

33 Lao WJ, Tsukada D, Greenstein DJ, Bay SM and Maruya KA, Analysis, occurrence, and toxic potential of pyrethroids, and fipronil in sediments from an urban estuary. *Environ Toxicol Chem* **29**:843–851 (2010).

34 Weston DP, Holmes RW and Lydy MJ, Residential runoff as a source of pyrethroid pesticides to urban creeks. *Environ Pollut* **157**:287–294 (2009).

35 Greenberg L, Rust MK, Richards J, Wu XQ, Kabashima J, Wilen C *et al.*, Practical pest management strategies to reduce pesticide runoff for

Argentine ant (Hymenoptera: Formicidae) control. *J Econ Entomol* **107**:2147–2153 (2014).

36 CA Department of Pesticide Regulation, *Nonfumigant Volatile Organic Compound (VOC) Regulations Product list*. http://www.cdpr.ca.gov/docs/emon/vocs/vocproj/nonfum_voc_prod_list.pdf [updated September 7, 2016].

37 Nelson EH and Daane KM, Improving liquid bait programs for Argentine ant control: bait station density. *Environ Entomol* **36**:1475–1484 (2007).

38 Klotz JH, Rust MK, Gonzalez D, Greenberg L, Costa H, Phillips P *et al.*, Directed sprays and liquid baits to manage ants in vineyards and citrus groves. *J Agric Urban Entomol* **20**:31–40 (2003).

39 Greenberg L, Tollerup KE and Rust MK, Control of Argentine ants (Hymenoptera: Formicidae) in citrus using methoprene and imidacloprid delivered in liquid bait stations. *Fla Entomol* **96**:1023–1029 (2013).

40 Cooper ML, Daane KM, Nelson EH, Varela LG, Battany MC, Tsutsui ND *et al.*, Liquid baits control Argentine ants sustainably in coastal vineyards. *Calif Agric* **62**:177–183 (2008).

41 Klotz JH, Rust MK, Field HC, Greenberg L and Kupfer K, Low impact directed sprays and liquid baits to control Argentine ants (Hymenoptera: Formicidae). *Sociobiology* **54**:101–108 (2009).

42 Rust MK, Reierson DA and Klotz JH, Delayed toxicity as a critical factor in the efficacy of aqueous baits for controlling Argentine ants (Hymenoptera: Formicidae). *J Econ Entomol* **97**:1017–1024 (2004).

43 Rust MK, Soeprono A, Wright S, Greenberg L, Choe DH, Boser CL *et al.*, Laboratory and field evaluations of polyacrylamide hydrogel baits against Argentine ants (Hymenoptera: Formicidae). *J Econ Entomol* **108**:1228–1236 (2015).

44 Buczkowski G, Roper E and Chin D, Polyacrylamide hydrogels: an effective tool for delivering liquid baits to pest ants (Hymenoptera: Formicidae). *J Econ Entomol* **107**:748–757 (2014).

45 Buczkowski G, Roper E, Chin D, Mothapo N and Wossler T, Hydrogel baits with low-dose thiamethoxam for sustainable Argentine ant management in commercial orchards. *Entomol Exp Appl* **153**:183–190 (2014).

46 Holliman PJ, Clark JA, Williamson JC and Jones DL, Model and field studies of the degradation of cross-linked polyacrylamide gels used during the revegetation of slate waste. *Sci Total Environ* **336**:13–24 (2005).

47 World Health Organization, *International Programme on Chemical Safety: Environmental Health Criteria No. 49: Acrylamide*. WHO, Geneva, pp. 1–121 (1985).

48 Slayne MA and Lineback DR, Acrylamide: considerations for risk management. *J AOAC Int* **88**:227–233 (2005).

49 Zovko M, Vidaković-Cifrek Z, Cvetković Z, Bošnir J and Šikić S, Assessment of acrylamide toxicity using a battery of standardised bioassays. *Arh Hig Rada Toksikol* **66**:315–321 (2015).

50 International Agency for Research on Cancer, Acrylamide, in *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. IARC, Lyon, France, pp. 389–433 (1994).

51 Murata Y, Jinno D, Kofuji K and Kawashima S, Properties of calcium-induced gel beads prepared with alginate and hydrolysates. *Chem Pharm Bull* **52**:605–607 (2004).

52 Işıklan N, Controlled release study of carbaryl insecticide from calcium alginate and nickel alginate hydrogel beads. *J Appl Polym Sci* **105**:718–725 (2007).

53 Roy A, Bajpai J and Bajpai AK, Dynamics of controlled release of chlorpyrifos from swelling and eroding biopolymeric microspheres of calcium alginate and starch. *Carbohydr Polym* **76**:222–231 (2009).

54 Saarai A, Kasparkova V, Sedlacek T and Saha P, A comparative study of crosslinked sodium alginate/gelatin hydrogels for wound dressing, in *Proceedings of the 4th WSEAS International Conference on Engineering Mechanics, Structures, Engineering Geology*, ed. by Mastorakis N, WSEAS Press, Greece, pp. 384–389 (2011).

55 Wang YF, Liu MZ, Ni BL and Xie LH, K–carrageenan–sodium alginate beads and superabsorbent coated nitrogen fertilizer with slow-release, water-retention, and anticompaction properties. *Ind Eng Chem Res* **51**:1413–1422 (2012).

56 Kulkarni AR, Soppimath KS, Aminabhavi TM and Dave AM, Polymeric sodium alginate interpenetrating network beads for the controlled release of chlorpyrifos. *J Appl Polym Sci* **85**:911–918 (2002).

57 Tonnesen HH and Karlsen J, Alginate in drug delivery systems. *Drug Dev Ind Pharm* **28**:621–630 (2002).

58 Klotz JH and Shorey HH, Low-toxic control of Argentine ants using pheromone-enhanced liquid baits. *California Department of Consumer Affairs, CDCA 84SA8020-07* (2000).

59 SPSS Inc, *SPSS user's guide*, version 11.5 for Windows, SPSS Inc., Chicago, IL (2002).

60 Zar JH, Data transformations, in *Biostatistical Analysis*, 4th edition. Prentice Hall, Upper Saddle River, NJ, pp. 271–281 (1999).

61 Byrne FJ, Toscano NC, Urena AA and Morse JG, Quantification of imidacloprid toxicity to avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), using a combined bioassay and ELISA approach. *Pest Manag Sci* **61**:754–758 (2005).

62 Markin GP, Food distribution within laboratory colonies of Argentine ant, *Iridomyrmex humilis* (Mayr). *Insectes Soc* **17**:127–158 (1970).

63 Reierson DA, Rust MK and Hampton-Beesley J, Monitoring with sugar water to determine the efficacy of treatments to control Argentine ants, *Linepithema humile* (Mayr), in *Proceedings of the National Conference on Urban Entomology*, San Diego, *CA*, pp. 78–82 (1998).

64 Choe DH and Rust MK, Horizontal transfer of insecticides in laboratory colonies of the Argentine ant (Hymenoptera : Formicidae). *J Econ Entomol* **101**:1397–1405 (2008).

65 Smidsrød O and Draget KI, Alginate gelation technologies, in *Food Colloids: Proteins, Lipids and Polysaccharides*, ed. by Dickinson E and Bergenstahl B, Royal Society of Chemistry, Cambridge, UK, pp. 279–293 (1996).

66 Dimonie D, Petrache M, Trusca R, Vasile E, Dinescu SM and Fierascu RC, Modulation of ionotropic alginate hydrogel microarchitecture by controlling the crosslinker ions migration. *Dig J Nanomater Bios* **8**:11–24 (2013).

67 Walters AC and Mackay DA, An experimental study of the relative humidity preference and survival of the Argentine ant, *Linepithema humile* (Hymenoptera, Formicidae): comparisons with a native *Iridomyrmex* species in South Australia. *Insectes Soc* **50**:355–360 (2003).

68 Abril S, Oliveras J and Gómez C, Effect of temperature on the development and survival of the Argentine ant, *Linepithema humile. J Insect Sci* **10**:1–13 (2010).