Laboratory and Field Evaluations of Polyacrylamide Hydrogel Baits Against Argentine Ants (Hymenoptera: Formicidae)

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ABSTRACT The development of effective baits to control the Argentine ant, *Linepithema humile* (Mayr), has been problematic because foragers prefer sweet liquids, while many toxicants are insoluble in water and liquid baits are generally difficult to deliver. The incorporation of thiamethoxam and sucrose solutions into a water-absorbing polyacrylamide hydrogel provides a unique and novel carrier and method of application for liquid baits. Formulations of thiamethoxam affected the size of the hydrogels, and sucrose solutions containing 0.0003% technical thiamethoxam provided hydrogels as large as those made with 25% sucrose solution or deionized water. Concentrations of thiamethoxam as low as 0.000075% in the hydrogels provided 50% kill of workers within 3 d in a laboratory setting. In small colony studies, baiting with 0.00015 and 0.000075% thiamethoxam hydrogels provided 100% mortality of workers and queens within 8 d. An enzyme-linked immunosorbent assay indicated that thiamethoxam was absorbed into the interior of the polyacrylamide matrix. The water loss rates of the hydrogels were dependent upon the relative humidity. Polyacrylamide hydrogels with >50% water loss were less attractive to ants. Field studies in highly infested areas indicated that concentrations of 0.0006 or 0.0018% thiamethoxam were more effective than 0.00015%. Hydrogels may provide a cost-effective alternative to providing aqueous baits to control Argentine ants.

KEY WORDS *Linepithema humile*, thiamethoxam, polyacrylamide hydrogel

Baits have been recommended for Argentine ant, *Linepithema humile* (Mayr), control for nearly a century with limited success (Rust 2001, Klotz et al. 2008, Silverman and Brightwell 2008). Liquid sucrose baits containing boric acid, imidacloprid, indoxacarb, and thiamethoxam have shown promise in agricultural and urban settings (Danne et al. 2006, 2008; Greenberg et al. 2006; Klotz et al. 2009). However, one of the major disadvantages with liquid baits has been the cost associated with applying and maintaining bait stations. Klotz et al. (2009) found that the use of liquid bait stations containing 0.001% thiamethoxam provided satisfactory control in residential settings, but increased the cost of the treatments by 40% compared with conventional spray treatments. The costs of baits and stations were also an issue in controlling *L. humile* in vineyards (Danne et al. 2008).

Gel bait formulations have revolutionized the control of German cockroaches, *Blattella germanica* (L.) (Rust 2001, Schal 2011), but similar attempts to extend this technology to controlling *L. humile* have met with limited success. Silverman and Roulston (2001) found that *L. humile* preferred liquids compared with gel baits and that gel baits increased the handling time of workers at the bait stations. In field studies with gel baits containing indoxacarb and thiamethoxam, *L. humile* actively fed on baits, but it was not possible to deliver sufficient quantities of bait to provide meaningful and cost effective control (M.K.R., unpublished data). In an attempt to eliminate *L. humile* on Santa Cruz Island off the California coast in 2012, researchers at The Nature Conservancy incorporated toxicants and sucrose into an inexpensive water-storing crystals (polyacrylamide hydrogels, Boser et al. 2014) used by horticulturalists to maintain soil moisture (Johnson 1984, Orzolek 1993). Buczkowski et al. (2014a) demonstrated that these hydrogels containing 0.0007% thiamethoxam (it was erroneously calculated as 0.007%) provided kill of Argentine ants in laboratory colonies.

The objectives of this study were to incorporate sucrose and thiamethoxam in the water-storing hydrogels and assess their effectiveness as a bait delivery system. The optimal concentration of thiamethoxam was assessed in a laboratory and a field setting. The rate of water loss, the distribution of toxicant in the polyacrylamide hydrogels, and the attractiveness of the hydrogels to workers as they desiccated were determined. The potential of this type of novel bait matrix is discussed.

Materials and Methods

Toxicant. Thiamethoxam was selected as the toxicant because it consistently provided kill of queens in laboratory studies, was partly water soluble (4.1 g/liter),

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and readily dissolved in 25% sucrose solutions at concentrations required to kill ants (Rust et al. 2004). In the initial laboratory studies, an experimental liquid bait consisting of 0.003% thiamethoxam (Syngenta Crop Protection, Greensboro, NC) was diluted and used to condition the crystals. In later laboratory studies, either 0.01% ant gel (Optigard Ant Gel Bait, Syngenta Crop Protection) or technical thiamethoxam was diluted with 25% sucrose water. We used deionized water in the laboratory (pH ~ 6.0) to mix the sucrose solutions. Field studies used thiamethoxam (Optigard Flex Liquid, Syngenta Crop Protection) diluted with groundwater on site to prepare the hydrogels.

Preparation of Polyacrylamide Hydrogels. In the laboratory studies, crystals (Miracle-Gro Water-Storing Crystals, The Scotts Company LLC, Marysville, OH) were individually weighed and placed into 59-ml plastic soufflé cups (Amerifoods Trading Co., Los Angeles, CA). Approximately, 15 ml of water, 25% sucrose solution, or 25% sucrose solution with diluted thiamethoxam were poured into each cup. The cups were covered with a lid and placed in the refrigerator for at least 18 h. The hydrogels were stored in the refrigerator until used.

In field studies, two sizes of hydrogel crystals were hydrated with the thiamethoxam and sucrose solution until saturated for at least 24 h. The small crystals, also used in laboratory studies, are about 0.75 cm^3 when fully hydrated. We also tested large spherical hydrogels (Deco Beads, JRM Chemical, Cleveland, OH) which are 3.5 cm^3 when fully hydrated.

Continuous Exposure Test. To determine the inherent toxicity of hydrogels impregnated with thiamethoxam, worker ants were continuously confined with the hydrogels in small plastic petri dishes (Rust et al. 2004). Concentrations of 0.003, 0.0003, 0.00015, 0.000075, and 0.0000375% thiamethoxam (wt/vol) were tested. One small vial (8 mm diameter by 30 mm) was filled with the 0.2 cc of hydrogel bait and one was filled with 25% sucrose water. Both vials were placed in the bottom of each plastic petri dish (50 mm diameter by 15 mm). Ten workers were selected from colonies that had been deprived of sugar water and food for 72 h and placed in the petri dish and the dish was covered with a lid. The dishes were placed in an environmental chamber maintained at 100% relative humidity (RH).

The number of dead workers was counted daily for 7 d. All concentrations were replicated 10 times. The continuous exposure data were analyzed with a Kaplan–Meier Survival function test (Statistix 2013) to determine the survivorship function ($S_{(t)}$) and survivorship percentiles (SPs) for each treatment. This test accounts for right censored data or individuals not dead at the termination of the test. The $S_{(t)}$ is the probability that ants will be alive at time *t*. The SPs indicate the day at which a given number of ants will be alive. Both allow the different treatments to be statistically compared with one another.

Laboratory Small Colony Test. To determine the efficacy of the toxicant-laced hydrogels against Argentine ants, 0.00015 and 0.000075% thiamethoxam hydrogel baits were placed in laboratory colonies to determine if they killed workers and queens. Colonies containing about 300 workers + a mix of eggs and larvae (10–15) + 1 queen were set up in plastic boxes (17.8 by 11.4 by 10.2 cm^3) with the inside walls coated with a thin film of Teflon emulsion to prevent ants from escaping. The colonies were provided a plastic petri dish (8.5 cm) partially filled with hardened plaster of Paris and covered with dark cardboard to serve as a nesting site for the ants. The plaster was moistened to humidify the nest. The containers were provisioned with a 25-ml water vial, a plastic dish containing dead American cockroaches, *Periplaneta americana* (L.), and a dish of 25% sucrose water. The colony boxes were placed inside sealed chambers maintained at 42% RH (saturated $MgCl_2$ solution) or at 75% RH (saturated NaCl solution, Winston and Bates 1960) throughout the study. The colonies acclimated for about 7 d before testing.

The sugar water and cockroaches were removed from the food containers about 72 h prior to baiting. Studies by Markin (1970) showed that starvation of laboratory colonies for 72 h simulated the intensity of hunger and foraging of field populations. A small piece of bait (0.1 g) for each concentration was placed in each of three test colonies. At 24 h posttreatment, sugar water and pieces of dead American cockroaches were provided to the colonies. The number of dead workers and queens was counted daily until all the ants were dead in the treatment.

Amount of Thiamethoxam in the Hydrogels. To determine if thiamethoxam migrated into the hydrogel matrix, the amount of thiamethoxam on the surface or inside the gels was determined using an enzyme-linked immunosorbent assay (ELISA). Polyacrylamide hydrogels were prepared as described above in 15 ml of 0.000075% thiamethoxam (Optigard ant gel) in 25% sucrose water. Control hydrogels were prepared in a 25% sucrose solution. After 18 h, the hydrogels were removed from the solution and briefly touched with a Kimwipe tissue (Kimberly-Clark Professional, Roswell, GA) to remove any excess liquid from the surface. A clean razor blade was used to trim about 2–3 mm from the outside of the hydrogel leaving a small inner cube. The blade was cleaned in between each cut. The small cube weighed about 0.05 g. The trimmed sections of the crystal also weighed about 0.05 g. The small cube and the trimmed portions were placed into separate 1.5-ml centrifuge tubes. Exactly 0.3 ml of distilled water was added to each sample. The samples were homogenized with a plastic pestle. After centrifuging for 5 min, 20 µl of supernatant was carefully removed and diluted in 980 µl of distilled water (50-fold dilution) to minimize the matrix effect. The matrix effect, broadly defined as interference with the analytical technique by one or more constituents of the sample (e.g., hydrogel and sucrose in the current study), can lead to a consistent bias in analysis determinations (Wild 2005). To determine the amount of thiamethoxam incorporated in the crystal, an ELISA technique similar to Byrne et al. (2005, 2007) and Ma et al. (2009) was used. The ELISA kit is available commercially (Thiamethoxam H.S. Plate Kit, catalog no. 20-0102, Beacon Analytical

Systems Inc., Saco, ME) with a 0.05–2 µg thiamethoxam liter^{-1} sensitivity range. Sixty microliters of the diluted sample were transferred into a 96-well microplate. Sixty microliters of thiamethoxam HRP (horseradish peroxidase) conjugate and 60 µl of the antibody solutions were pipetted into the microplate and mixed for 30 s with a plate mixer. To initiate the reaction, 150 µl of the mixtures were pipetted out and put in the ELISA test strips. After 60-min incubation at ambient temperature, the excess of the preparation from the ELISA test strips was disposed, and the test strips were washed with clean water. One hundred microliters of substrate were added into each well of the ELISA test strip. The reaction was stopped with 100 µl of the stop solution (HCl) after 30-min incubation. The optical density was read at 450 nm.

The estimated amount of thiamethoxam inside and outside the hydrogel cube was analyzed with a paired *t*-test (Statistix 2013).

Effect of RH on Water Loss of Hydrogels. Hydrogels were weighed and conditioned as described above in 15 ml of deionized water, 25% sucrose, or 25% sucrose plus thiamethoxam solutions. After soaking in the solutions for 18 h, each hydrogel was removed and placed in a weighing boat. The excess liquid on the surface of each hydrogel was removed with a Kimwipe tissue before weighing. The hydrogels were then placed in a 5-liter desiccator containing saturated salt solutions at 25.6°C providing 0–2% RH (anhydrous $CaSO_4$), 32% RH ($MgCl_2{\cdot}6H_2O$), 52% RH (Na_2CrO_4), or 75% RH (NaCl; Winston and Bates 1960). Each hydrogel was weighed after 2, 4, 6, 8, and 24 h. To determine the percent water loss rates, the hydrogels were then placed into a desiccator maintained at 25.6°C and 0–2% RH (anhydrous $CaSO_4$). The hydrogels were weighed every 2–3 d until there were similar successive weights and all the water had been lost.

The total amount of water absorbed by the crystals was analyzed with an ANOVA and the means were separated with a Tukey's HSD test at $P < 0.05$. The gain in weight of hydrogels and the rate of water lost at each hour for the hydrogels were plotted as a nonlinear regression (Statistix 2013).

Choice Feeding Study with Partially Dehydrated Hydrogels. To determine how long the baits were attractive to ants as the hydrogels lost water, ants were given a choice of hydrogels with differing amounts of water loss. Small laboratory colonies of ants containing approximately 1,000–3,000 workers, queens, and brood were deprived of 25% sucrose water for about 72 h.

Crystals were conditioned in 25% sucrose water for 18 h. The hydrogels were removed and placed into desiccators maintained at 32% RH for 4.7, 12.3, and 26.9 h to provide hydrogels that had lost approximately 25, 50, and 75% of their water, respectively. About 0.2 cc of the conditioned hydrogels were placed in the caps. Workers were provided a choice of hydrogels with 0, 25, 50, and 75% water loss. The bait acceptance arena was constructed with a white hardboard square (9 by 9 cm^2), on which four small plastic cups (i.e., inverted centrifuge tube cap) were attached 3.5 cm away from the center at 90° intervals (Fig. 3). A digital picture was taken every 5 min for 75 min. The number of ants feeding on the hydrogels was recorded while there was foraging starting at 5 min and ending at 75 min.

The data from choice tests with desiccated hydrogels were analyzed with a repeated-measures ANOVA on the square-root transformed data with time as the repeating factor, percent evaporation of the hydrogel as the factor being tested, and boxes as the "subjects" or replicates. This was followed up with a simple ANOVA of each time period, means being separated with the Tukey's HSD test at $P < 0.05$ (Systat 2009).

Determining Optimal Concentration of Thiamethoxam in the Field. Santa Cruz Island, located in the Channel Islands National Park off the coast of Santa Barbara, CA, hosts a population of *L. humile* in a natural habitat (Boser et al. 2014). A randomized complete block design, with five complete blocks, was used to compare the efficacy of four bait treatments in a field setting. Each block consisted of four 30- by 30-m treatment plots and a 30- by 30-m control plot. Plots within each block were matched as closely as possible with respect to vegetation, aspect, and soil characteristics and were separated by ≥20 m to maintain plot independence. Three blocks were established within dominant fennel stands, and two blocks were established within oak habitat.

The small hydrogels were hydrated with a 25% sucrose solution with three different concentrations of thiamethoxam (0.00015, 0.0006, and 0.0018%). Only one concentration of 0.0006% thiamethoxam was tested with the larger hydrogels. Bait was applied to the treatment plots in two applications, from 10–11 August 2012 and 28–29 September 2012. The bait was dispersed at a rate of 4.5–5.7 liter of hydrogels per treatment plot (7.5 ml/m^2). The small hydrogels were dispersed by hand in small piles of approximately 10 ml on a 2- by 2-m grid covering the entire treatment plot, whereas the large hydrogels were uniformly dispersed by hand-tossing. No hydrogels were applied in the control plots. To measure treatment efficacy, the experimental and control plots were monitored for ant activity 1 d prior to bait deployment, and at 4 and 9 wks after the initial treatment. A 50-ml centrifuge tube filled with a 25% sucrose-saturated cotton ball was deployed every 5 m within the core 100 m^2 of each plot for a total of nine monitoring points per plot. After 1–2 h, the tubes were collected and the Argentine ants in the tube were counted.

The change in the mean Argentine ant abundance after treatment, relative to before treatment, was calculated as follows: $\%\ Pretreatment = [(\text{mean abundance posttreatment})/(\text{mean abundance pretreatment})] \times 100$. Variation in mean Argentine ant abundance randomized complete block field trails was analyzed with a blocked repeated-measures GLM, using the bait treatment as the fixed factor, block as the blocking factor, and time as the repeated-measures factor. To examine the mean Argentine ant abundance during the final monitoring round, a 2-way ANOVA with block and treatment as the main factors was performed. Paired

t-tests were performed for a posteriori comparisons because the blocking factor was significant. Bonferroni corrections for multiple comparisons were used to correct for type 1 error. To determine if there was an interaction between habitat and treatment efficacy, a two-way ANOVA was performed with habitat and treatment as the independent variables and percent pretreatment as the dependent variable. Prior to analysis the Argentine ant monitoring data were normalized using a $\log_{10}(x+1)$ transformation to account for the zeros in the dataset and the percent pretreatment data were normalized with an arcsine square root transformation. All statistical analyses were performed with Systat (2009).

Results

Toxicant and Preparation of Hydrogel Bait. The hydrogels increased in weight by 375- and 349-fold when placed in deionized water and 25% sucrose, respectively (Table 1). Hydrogels made with technical thiamethoxam were significantly smaller than those made in water or 25% sucrose ($F = 51.45$; df = 7,72; $P < 0.001$). When the thiamethoxam solutions were prepared from an experimental 0.01% ant gel bait, the hydrogels grew larger than they did in the diluted 0.003% thiamethoxam liquid ant bait. When placed in dilutions prepared from the 0.003% thiamethoxam liquid ant bait, the hydrogels were significantly smaller than those in water, 25% sucrose, technical thiamethoxam, and diluted ant gel.

As the concentration of thiamethoxam increased, the size of the hydrogels decreased in a nonlinear manner ($Y = 1/2.96 \times 10^{-3} + 5.8488X$, $R^2 = 0.954$, Fig. 1). When hydrogels were grown in serial dilutions of 0.00015% or less thiamethoxam in 25% sucrose, their size was the same as those in 25% sucrose solutions (Table 2). Hydrogels conditioned in 0.003 and 0.0003% thiamethoxam in 25% sucrose were significantly smaller.

Continuous Exposure Test. Baits containing 0.0003% or more thiamethoxam provided significantly faster kill of the ants than did lower concentrations, only 17% being alive at day 3 (Table 2). The 0.000075 and 0.00015% thiamethoxam hydrogels provided 50% survivorship at day 3, and these two concentrations were selected for testing in the small colony studies.

Laboratory Small Colony Test. The 0.00015% thiamethoxam hydrogels provided nearly 100% kill within 8 d when colonies were maintained at both 42 and 74% RH (Table 3). There was no significant difference in mortality at day 8 between the RHs ($F = 0.33$; df = 1,4; $P = 0.599$). The mortalities were 24.5% on day 1 and 88.4% on day 4. All the queens died within 8 d, the median being 4 d.

The 0.000075% thiamethoxam hydrogel baits provided 100% kill of workers and queens within 10 d at both 42% and 74% RH (Table 4). There were no significant difference in mortality of workers between 42% and 74% at day 5 ($F = 0.20$; df = 1,4; $P = 0.677$). At day 1, there was <5% mortality at either RH. On day 5, the overall mortality was 73.6% and 81.5% at 42% and 74% RH, respectively. All queens died by day 10 and the median value was 5.5 d.

Table 1. Increased growth ratio (final/initial) of hydrogels placed in various solutions of thiamethoxam and 25% sucrose

Solutions	Form[a]	Growth ratio (X ± SD)[b]
Deionized water		375.3 ± 63.69a
25% sucrose water		348.8 ± 64.76a
0.0003% thiamethoxam	Liq	132.3 ± 32.56c
0.003% thiamethoxam	Liq	40.0 ± 7.66d
0.0003% thiamethoxam	Gel	198.6 ± 40.31bc
0.003% thiamethoxam	Gel	184.87 ± 12.09bc
0.0003% thiamethoxam	Tech.	237.0 ± 61.66b
0.003% thiamethoxam	Tech.	231.2 ± 58.06b

[a] liq—0.003% liquid ant bait; gel—Optigard Ant Gel; tech.—technical grade.
[b] Means followed by the same letter are not significantly different at $P < 0.05$ (Tukey's HSD). $n = 10$.

Migration of Thiamethoxam into the Hydrogels. Thiamethoxam migrated into the hydrogel matrix. The estimated amounts of thiamethoxam per gram of hydrogel were 591.3 ± 45.3 and 640.6 ± 81.2 ng (mean ± SEM) for the surface and interior of the hydrogel, respectively. They were not significantly different ($t = 1.31$, df = 4, $P = 0.26$). Even though control hydrogels did not contain any thiamethoxam, low levels of absorbance (2.8 and 10.1 for inside and outside of the hydrogel, respectively) were obtained from the ELISA tests, suggesting a minor effect potentially caused by hydrogel and sucrose in the preparations.

Amount of Water Loss from Hydrogels. Water loss of hydrogels conditioned in deionized water was expressed as nonlinear regressions at each of the RHs: 0% RH ($y = 0.72 - 0.72 \times 0.96^x$); 33% RH ($y = 0.66 - 0.68 \times 0.93^x$); 55% RH ($y = 0.53 - 0.54 \times 0.94^x$); 75% RH ($y = 0.39 - 0.40 \times 0.93^x$). Water loss of hydrogels conditioned in 25% sucrose was expressed as nonlinear regressions at each of the RHs: 0% RH ($y = 1.30 - 1.30 \times 0.96^x$); 33% RH ($y = 0.92 - 0.90 \times 0.94^x$); 55% RH ($y = 0.55 - 0.55 \times 0.95^x$); 75% RH ($y = 0.60 - 0.61 \times 0.94^x$). Water loss of the hydrogels conditioned in 0.00015% thiamethoxam and sucrose solution was expressed as a nonlinear regression at each RH: 0% RH ($y = 1.30 - 1.29 \times 0.95^x$); 33% RH ($y = 1.21 - 1.17 \times 0.95^x$); 55% RH ($y = 0.61 - 0.58 \times 0.93^x$); 75% RH ($y = 0.59 - 0.58 \times 0.95^x$). The nonlinear regressions of the hydrogels conditioned in 0.000075% thiamethoxam and sucrose are shown in Figure 2.

The water loss rates for the hydrogels exposed to 52 and 75% RH and 0 and 32% RH were similar for those that contained 25% sucrose. Interestingly, the hydrogels conditioned in deionized water lost water at a slower rate than did the crystals in 25% sucrose or either of the thiamethoxam hydrogels. The times required for the hydrogels to lose 50% of the water content were calculated (Table 5). At the two low RHs, it will require 9–12 h before the thiamethoxam hydrogels lose 50% of their water. At the two higher RHs, it

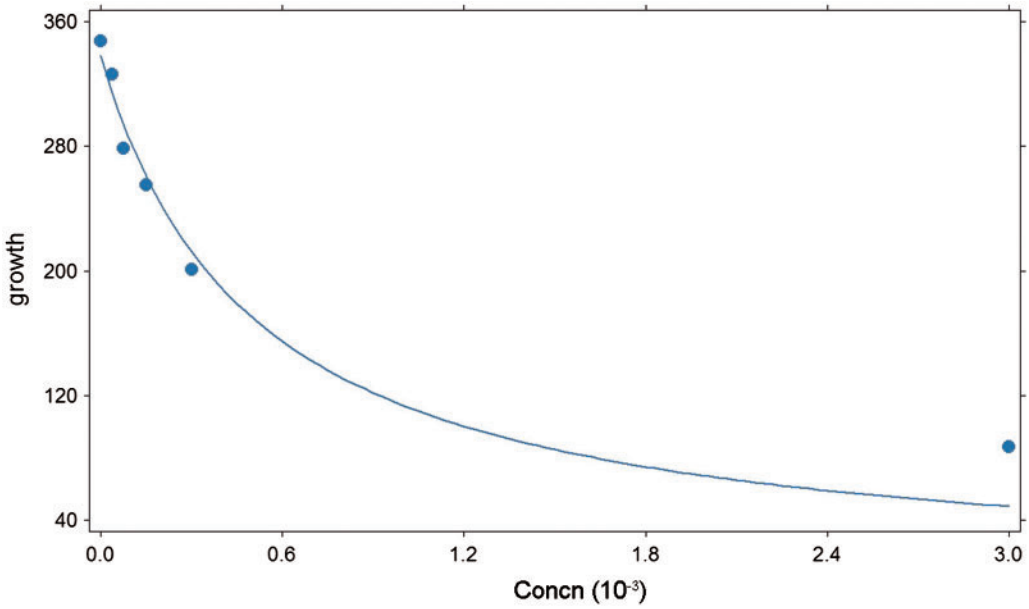

Fig. 1. Relationship between the concentration of thiamethoxam and the growth of the hydrogel crystals.

Table 2. Increased growth ratio (final/initial wt.) of hydrogels conditioned in various dilutions of thiamethoxam and their toxicity to *L. humile*

Solutions	n	Growth ratio X ± SD[a]	Survivorship function S(t) at [day 3] (95% CL)	Survivorship percentiles 50% at day
0.003%	10	86.9 ± 20.31c		
0.0003%	10	201.0 ± 35.82b	0.17 (0.101–0.223)	2 (2–3)
0.00015%	10	255.0 ± 25.80ab	0.30 (0.246–0.367)	3 (3–3)
0.000075%	10	278.5 ± 104.30ab	0.59 (0.505–0.669)	3 (3–4)
0.0000375%	10	326.0 ± 36.90a	0.63 (0.546–0.701)	4 (4–4)
25% Sucrose	10	347.4 ± 66.47 a	0.91 (0.838–0.951)	M

[a] Thiamethoxam baits prepared from ant gel. Means followed by the same letter are not significantly different at $P < 0.05$ (Tukey's HSD).

Table 3. Small colony studies with 0.00015% thiamethoxam hydrogel baits maintained at two relative humidities[a]

Replicate	RH	Percent dead workers on day			Queen death (d)
		1	4	8	
A	42%	10.5	73.8	93.8	8
B		38.2	88.2	100	3
C		47.3	100		2
Control		3.4	9.6	32.6	
A	74%	11.5	89.9	94.0	5
B		33.5	88.5	100	4
C		5.9	90.1	100	4
Control		1.2	4.5	9.3	

[a] Colonies initially set up with 300 workers, 1 queen, and brood on 30 March 2012. The colonies were allowed to acclimate for about 7 d before testing.

Table 4. Small colony studies with 0.000075% thiamethoxam hydrogel baits maintained at two relative humidities[a]

Replicate	RH	Percent dead workers on day			Queen death (d)
		1	5	10	
A	38%	0.5	66.5	100	4
B		0	74.3	100	5
C		0	80.2	100	6
Control		0	5.6	6.4	
A	74%	0	47.2	100	10
B		3.8	98.3	100	8
C		5.2	99.2	100	3
Control		0	1.2	3.6	

[a] Colonies initially set up with 300 workers, 1 queen, and brood on 28 February and 16 March 2012.

will require 22–53 h for the hydrogels to lose 50% of their water.

Determining How Long the Hydrogels Are Attractive. When the hydrogels had lost >25% of their water, their attractiveness to foraging ants declined significantly (Fig. 4). The attractiveness of the hydrogels with 0 and 25% water loss gradually declined during the test because the small laboratory colonies were quickly satiated. Percent evaporation was significant overall ($F = 110.2$; df = 3, 16; $P < 0.001$). However, the interaction term, time × percent evaporation, was also significant ($F = 4.1$; df = 12, 64; $P < 0.001$), indicating that not all the crystals saturated

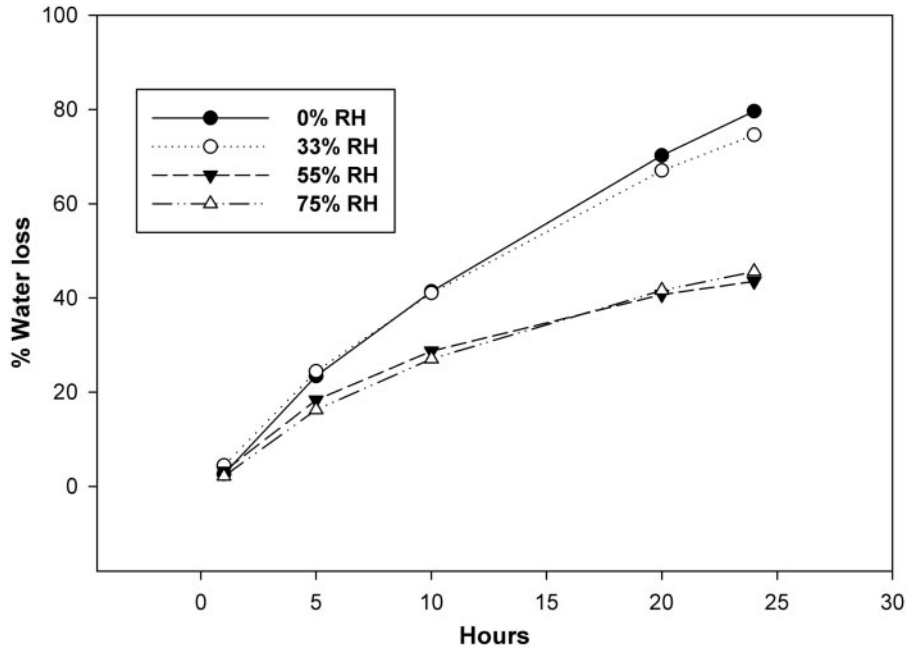

Fig. 2. Water loss of hydrogels conditioned in 0.000075% thiamethoxam gel bait solution. 0% RH, $y = 1.55 - 1.52 \times 0.97^x$; 33% RH, $y = 1.26 - 1.21 \times 0.96^x$; 55% RH, $y = 0.51 - 0.48 \times 0.93^x$; 75% RH, $y = 0.62 - 0.60 \times 0.95^x$.

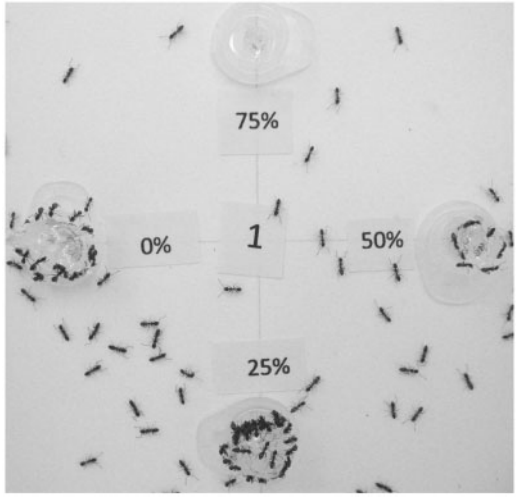

Fig. 3. Choice feeding arena with hydrogels that lost 0, 25, 50, and 75% of the water at 35 min.

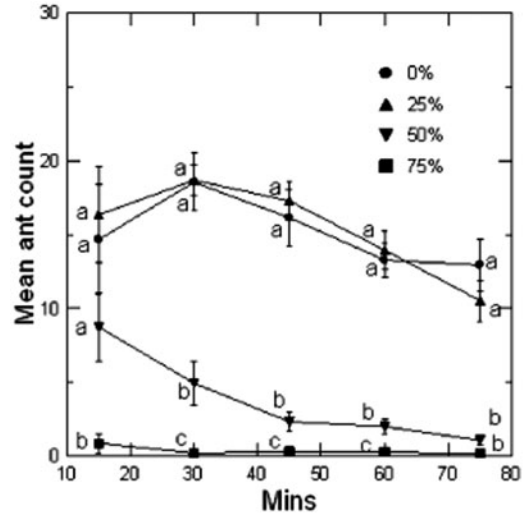

Fig. 4. Ant counts over time. Counts are the averages of three evenly spaced counts during 10 min ending at the shown data point. Legend shows percent evaporation of water from the hydrogels. For each time period, a different letter indicates a significant difference between the means (square-root transformed data, Tukey's HSD test, $P < 0.05$).

with different solutions had the same response over time.

Determining Optimal Concentration of Thiamethoxam in the Field. Prior to bait deployment, there was no significant difference in Argentine ant abundance within experimental plots ($F = 0.552$; df = 4, 20; $P < 0.699$). The change in Argentine ant abundance differed significantly between treatments through time (Argentine ant treatment × time GLM, $F = 2.652$; df = 12, 48; $P = 0.008$). The abundance of Argentine ants increased by an average of 266 ± 89% in the control plots 4 wks after the first treatment and by 87 ± 88% 1 wk after the second treatment, whereas in the treatment plots the abundance of Argentine ants increased by an average of 17 ± 20% 4 wks after the first treatment and decreased by 88 ± 3% 1 wk after the second treatment (Table 6). The abundance of

Table 5. The predicted water loss from hydrogels when held at various RHs

Hydrogels	RH (%)	% Water loss at hours			
		25	50	75	100
Water	0	9.47	28.59		
	33	6.97	19.94		
	55	10.61	46.71		
	75	12.13			
25% sucrose	0	5.23	11.89	21.07	35.92
	33	4.77	12.32	26.93	
	55	11.82	46.75		
	75	8.98	29.22		
0.00015% thiamethoxam	0	4.01	9.31	16.62	28.44
	33	3.86	9.74	18.20	33.49
	55	6.57	22.91		
	75	10.41	36.32		
0.000075% thiamethoxam	0	5.13	12.14	21.07	33.37
	33	4.43	11.39	21.16	37.67
	55	8.45	53.34		
	75	9.42	31.38		

Table 6. Mean (±SE) % pretreatment [(number of ants post-treatment/number of ants pretreatment) × 100] Argentine ant abundance within the five different treatment plots

Treatment	Days after treatment		
	24	49	57
Small hydrogel (0.00015% thiamethoxam)	137 ± 44%	105 ± 36%	17 ± 9%
Small hydrogel (0.0006% thiamethoxam)	139 ± 53%	54 ± 22%	13 ± 6%
Small hydrogel (0.0018% thiamethoxam)	81 ± 28%	43 ± 21%	10 ± 4%
Large hydrogel (0.0006% thiamethoxam)	112 ± 43%	34 ± 12%	7 ± 2%
Control	366 ± 89%	190 ± 74%	187 ± 20%

Argentine ants within the plots at the final monitoring round were significantly different between treatments ($F = 4.629$; df = 4, 16; $P = 0.011$). The abundance of Argentine ants in plots treated with small polymers (0.0006% and 0.0018%) and large polymers (0.0006%) was significantly lower than that in the control plots ($P > 0.013$ in all cases), whereas there was no significant difference between the plots treated with small polymer at a 0.00015% concentration and the control plots ($P = 0.082$). There was no significant difference in the treatment efficacy (i.e., % pretreatment) at the final monitoring round between the four baits tested ($P > 0.516$) and no significant interaction between treatment efficacy and habitat type (fennel and oak; $F = 1.491$; df = 4, 15; $P = 0.255$).

Discussion

There are a number of problems with developing aqueous baits for ant control or eradication. One is the cost associated with the baits and their delivery. Reuseable bait stations are costly and require labor to deploy and clean them. Many toxicants are not soluble in aqueous systems. Chemical companies are often reluctant to register toxicants as specialized baits for ants because of the limited market and cost constraints. In natural settings, it may be extremely difficult to place bait stations. In urban settings, professional pest management specialists are often reluctant to use ant bait stations when pets and children are present. Consequently, a novel delivery system is needed.

This study reports the use of polyacrylamide hydrogel matrix for developing novel liquid delivery system targeting pest ant species that would actively forage on sugary liquids. The current study also reports several important aspects of this novel use of polyacrylamide hydrogel. First, when workers fed on the surface of the polyacrylamide hydrogels, they ingested liquid and did not consume the hydrogel matrix. In contrast, when Argentine ants fed on many of the commercial ant gel baits, they actually consumed the gel (M.K.R., unpublished data). Second, the concentration of thiamethoxam in the hydrogel matrix was similar between the surface and interior parts of the hydrogel bait, suggesting a continuous supply of thiamethoxam in the aqueous solution as the ants drink it from the hydrogel surface. Third, the hydrogel baits lost their attractiveness to ants when about 50% of the water was lost through desiccation. Fourth, hydrogel baits maintained their attractiveness for foraging ants for 9 h at 0–2% RH and 36 h at 75% RH, suggesting the initial longevity (i.e., the window of time during which the bait maintains its attractiveness and palatability) of hydrogel bait will vary depending upon the RH in the environment.

The degree of expansion of the hydrogel in the aqueous bait preparation was significantly influenced by the type of thiamethoxam formulation. In general, conditioning the hydrogels in the sugar solution with formulated thiamethoxam (liquid and gel bait) reduced the size of final hydrogel baits compared with similar hydrogel baits conditioned in the sugar solution. However, the amounts of active ingredient required are so small that mixing batches of bait solutions with the technical material was problematic. Furthermore, when polyacrylamide hydrogels were conditioned in 25% sucrose solutions containing 0.0000375 to 0.00015% thiamethoxam (from ant gel bait), there was not an appreciable difference in size of the resultant hydrogel baits. Consequently, it was more practical to use the formulated ant gel bait to make the diluted thiamethoxam liquid bait to condition hydrogels compared with using the technical thiamethoxam. The use of thiamethoxam 2SC preparation for the field studies was preferred to prepare the hydrogel baits because the cost of the ant gel bait is considerably higher than that of thiamethoxam 2SC, and the field study involved much larger areas to be treated compared with the laboratory studies.

Our laboratory studies indicated that low concentrations of thiamethioxam provided in the hydrogel bait matrix were toxic to workers and queens, corroborating studies by Buczkowski et al. (2014a,b). Buczkowski et al. (2014a,b) reported that the hydrogel baits containing 0.007% thiamethoxam were toxic in both laboratory and field studies. However, they miscalculated the thiamethoxam concentration, which was actually 0.0007% and more similar to our data. Rust et al.

(2004) also previously reported that 0.0005–0.00001% thiamethoxam baits provided queen mortality in laboratory colony studies.

Field tests on Santa Cruz Island found that there was not a significant difference between the thiamethoxam concentrations tested (0.00015, 0.0006, and 0.0018%); however, the 0.00015% treatment was the only treatment not significantly different from the control. In the field, the 0.00015% concentration thiamethoxam may not be as effective as baits with higher concentrations because the toxicant dilution resulting from trophallaxis (i.e., food exchange) may prevent the toxicant from impacting the entire nest. The 0.0006 and 0.0018% treatments were equally effective at reducing Argentine ant populations; therefore, 0.0006% baits should be used in a control strategy to minimize the quantity of toxicant used and the environmental impact of the toxicant. This corroborates the findings of Buczkowski et al. (2014b) that 0.0007% thiamethoxam hydrogels provided 94% reduction of *L. humile* workers within 14 d.

We found no interaction between habitat type and hydrogel size or thiamethoxam concentrations chosen for the field study. The size of the hydrogel did not change the efficacy or the attraction of the hydrogel baits; however, the larger hydrogel baits were logistically easier to apply in the field. Furthermore, the relative efficacy of treatments did not change based on habitat type (i.e., fennel and oak). This result indicates that the use of hydrogel bait would be equally effective for those two dominant habitat types within the infested area of Santa Cruz Island.

The use of liquid bait, in conjunction with an effective delivery system, can dramatically reduce the use of pesticides while achieving effective control. For example, in our field study, the amount of active ingredient of thiamethoxam in hydrogels applied per square meter was 0.044 mg at the highest concentration applied. When thiamethoxam is applied to the soil in grapes (Platinum insecticide), it is applied at rates between 14.0 to 29.8 mg/m^2 (Syngenta 2014). There is at least a 318-fold reduction in the amount of thiamethoxam applied to the soil on Santa Cruz Island. Similarly, Buczkowski et al. (2014b) applied 150 g of the 0.0007% thiamethoxam hydrogels per 510 m^2 of a plum orchard. This is approximately 0.002 mg of thiamethoxam per square meter. Extremely low doses of thiamethoxam were effective when delivered in the hydrogels.

Scattering hydrated polyacrylamide hydrogels might be an extremely cost-effective approach to delivering sufficient quantities of aqueous liquid baits in agricultural, urban, or natural areas to manage some pest ant species. Along with its extremely low mammalian toxicity (i.e., LD_{50} oral for a rat is > 5.0 g/kg), the polyacrylamide hydrogel bait eliminates the need to check, clean, and refill bait stations. While the hydrogels are not biodegradable and do remain in the soil, they are only visible for a few days. Because the goal of this treatment is eradication, the number of treatments in one area is finite and hydrogels are not expected to build up in the environment. Researchers at TNC have already begun scattering these types of baits from aircraft in rugged and inaccessible natural areas of Santa Cruz Island, CA, with substantial success in controlling the invasive Argentine ant populations in those habitats (Boser et al. 2014).

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