Polyacrylamide Hydrogels: An Effective Tool for Delivering Liquid Baits to Pest Ants (Hymenoptera: Formicidae)

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ABSTRACT Ant management in urban and natural areas often relies on toxic baits. Liquid baits are highly attractive to pest ants because they mimic natural food sources such as honeydew and nectar, the principal dietary components of many ants. However, liquid bait use has been limited owing to the lack of bait dispensers that are effective, inexpensive, and easy to service. The current study evaluated the potential of water-storing crystals (polyacrylamide spheres) to effectively deliver liquid thiamethoxam baits to laboratory colonies of Argentine ants, *Linepithema humile* Mayr. Results of laboratory trials show that bait crystals saturated in 25% sucrose solution containing 0.007% thiamethoxam are highly attractive to Argentine ants and highly effective against all castes and life stages, including workers, queens, and brood. Fresh bait crystals were highly effective and required ≈2 d to kill all workers and ≈6 d to achieve complete mortality in queens and brood. Results of bait aging tests show that the crystals lose ≈70% of moisture in 8 h and the duration of outdoor exposure has a significant effect on moisture loss and subsequently bait acceptance and bait efficacy. A gradual decrease in mortality was observed for all castes and life stages as bait age increased. In general, fresh baits and those aged for <8 h retained their efficacy and caused substantial mortality. Baits aged longer than 8 h were substantially less attractive and less effective. Horizontal transfer tests examined the transfer of thiamethoxam from live treated donors to live untreated recipients. The results show that donor ants that obtain thiamethoxam by feeding on bait crystals effectively transfer it to untreated recipient ants. The level of secondary mortality depended on the donor:recipient ratio, with ≈40% recipient worker mortality with the 1:5 ratio and 15% recipient worker mortality with 1:10 or 1:20 ratios. However, no queens died in any transfer tests, suggesting that multiple feedings from multiple donors may be necessary to produce queen mortality. The results of the transfer tests demonstrate the role of trophallaxis in the distribution of thiamethoxam and confirm that thiamethoxam is effectively transferred in Argentine ant colonies. The distribution of thiamethoxam within Argentine ant colonies was further examined using protein marking coupled with an enzyme-linked immunosorbent assay to detect the marker. The distribution of thiamethoxam was highly efficient, with 79 ± 13% of workers testing positive at 15 min and 100 ± 0% of workers testing positive at 6 h. In summary, the results of this study demonstrate that water-storing crystals effectively deliver thiamethoxam to all castes and life stages of Argentine ants and may offer an effective tool for Argentine ant management.

KEY WORDS Argentine ant, bait, *Linepithema humile*, polyacrylamide, protein marking

The current options for the management of pest ants in urban, agricultural, and natural environments include the use of residual sprays or baits. Residual sprays are often highly effective but suffer a number of disadvantages. The main disadvantage is that residual sprays provide temporary control but have little long-term impact. This is because sprays only kill the foraging workers but have little effect on reproductives and workers that are often protected within subterranean nests (Knight and Rust 1990, 1991). This necessitates frequent reapplications, which are labor intensive and costly. Another drawback of residual insecticides is that they kill a significant proportion of nontarget beneficial organisms (Smith et al. 1996). This is a concern in agricultural and natural situations, where protecting the nontarget organisms is often as important as killing the unwanted ones. Finally, the relatively long residual activity of some sprays is a concern with regards to environmental pollution of soil and water, and pesticide residues in crops.

Toxic baits that exploit the recruitment and food-sharing behavior of ants are often used as an alternative to residual sprays. Baits reduce the need for broadcast sprays and therefore limit the amount of insecticide used to control ants (Hooper-Bui and Rust 2000). Baits also reduce nontarget and environmental effects because they require relatively small amounts

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of the active ingredient. Baits are typically applied as granules or liquids. Granular baits are protein- or oil-based and are mostly effective against protein-feeding myrmicine ants, such as fire ants and big-headed ants (Taniguchi et al. 2005), but are only weakly attractive to ants that feed mainly on liquid sugars such as honeydew or nectar and require liquid baits. Liquid baits are typically attractive to a wide range of ants, but suffer other drawbacks. One disadvantage is their relatively short life span under field conditions. Because of their high sugar content, liquid baits are highly susceptible to water loss by evaporation and contamination by yeast, which leads to fermentation and loss of palatability. Another disadvantage is the lack of commercially available bait dispensers that are effective, inexpensive, and easy to service. Currently available bait dispensers are labor intensive because they need to be put out in fairly large densities, require periodic cleaning and maintenance, and need to be frequently monitored and refilled (Nelson and Daane 2007).

To overcome the problems associated with traditional baits, a novel bait delivery technology that relies on water-storing crystals was tested on 20 acres of wildland habitat in southern California by Boser et al. 2014 who showed that water-hydrating crystals containing thiamethoxam at 0.0006 and 25% sugar water reduced Argentine ant activity by 99.8% relative to control plots within 12 mo. Water-storing crystals, also known as hydrogels, are superabsorbent polymers that are widely used in forestry, horticulture, and landscaping as a means of conserving water. The crystals are a cross-linked polyacrylamide copolymer gel and are able to absorb ≈300 times their weight in water. Dry granules resemble salt crystals and when immersed in water swell up and turn into a gel-like substance that resembles chunks of ice. The hydrated granules, called water crystals, slowly release water over time and the process may be repeated indefinitely. The crystals may also be used to absorb liquid insecticide baits for use in urban, agricultural, and wildland applications. The saturated water crystals combine the advantages of both sprays and liquid baits because they are easy to apply, do not require a dispenser, do not need to be serviced or refilled, are relatively inexpensive, and minimize insecticide use because they require a relatively small amount of active ingredient.

The current study evaluated the potential of water-storing crystals to effectively deliver liquid baits to laboratory colonies of Argentine ants, *Linepithema humile* Mayr. The Argentine ant is an invasive species that has successfully spread around the world with damaging ecological and economic consequences (Holway et al. 2002, Buczkowski et al. 2004, Roura-Pascual et al. 2004). It is a serious pest in natural, agricultural, and urban environments (Holway et al. 2002). In natural environments, Argentine ants outcompete native ants and other invertebrates, resulting in community-wide effects (Suarez et al. 2000). In urban environments, Argentine ants often reach extremely high densities and become a major nuisance when entering buildings in search of food, water, and shelter (Rust and Knight 1990, Klotz et al. 2002). In agricultural systems, Argentine ants are often associated with outbreaks of phloem-feeding homopterans such as aphids, scales, and mealybugs (Vega and Rust 2001, Daane et al. 2006), which interfere with biological control of honeydew-producing pests and leads to secondary problems such as sooty mold and transmission of plant viruses (Prins et al. 1990, Buckley and Gullan 1991).

Argentine ants feed predominantly on liquid foods such as honeydew and nectar (Markin 1970) and require a liquid bait for most effective control. Previous studies show that a variety of insecticides including boric acid, fipronil, imidacloprid, and thiamethoxam may be used in liquid baits for control of Argentine ants (Hooper-Bui and Rust 2000, Klotz et al. 2003, Daane et al. 2008). Liquid insecticide baits have proven effective for managing Argentine ants in agricultural situations including vineyards and citrus groves (Klotz et al. 2003; Daane et al. 2006, 2008). However, a major challenge that precludes a wider adoption of liquid baits in Argentine ant management is the lack of effective, economically feasible, and commercially available bait stations (Cooper et al. 2008).

The goal of the current study was to evaluate water-storing crystals as a tool to effectively deliver ultralow dose thiamethoxam baits to Argentine ants. If effective, water-storing crystals offer the potential to effectively deliver liquid baits for control of ants. The study had four main objectives: 1) test the ability of water-storing crystals to effectively deliver thiamethoxam to all castes and life stages of Argentine ants, 2) estimate the attractiveness and efficacy of thiamethoxam when delivered via crystals aged under outdoor conditions, 3) evaluate the horizontal transfer of thiamethoxam from fed donors to unfed recipients and the effect of donor:recipient ratio on bait distribution and efficacy, and 4) use protein marking to examine bait distribution in Argentine ant colonies. Taken together, the results of these four objectives advance our understanding of the benefits offered by water-storing crystals and contribute to the development of sustainable management tools for Argentine ants in natural and managed ecosystems.

Materials and Methods

Insects and Test Colonies. Colonies of Argentine ants, *L. humile,* were collected from a large polydomous supercolony in Winston-Salem, NC. The ants were transported to the Department of Entomology at Purdue University and extracted from the nesting material. Debris containing the ants was placed in plastic trays provided with moist plaster nests. As the debris dries, the ants moved into the plaster nests. Subsequently, colonies were maintained in debris-free Fluon-coated trays. Colonies were reared on 25% sucrose solution and artificial Bhatkar diet ad libitum (Bhatkar and Whitcomb 1970), hard-boiled egg, and freshly killed cockroaches once a week. Colonies were maintained and all experiments were conducted at 25 ± 2°C, 60 ± 10% relative humidity (RH), and a photoperiod of 14:10 (L:D) h.

Insecticide and Testing Substrates. To prepare the bait, Water Storing Crystals (100% polyacrylamide; Miracle Gro Lawn Products, The Scotts Company, Marysville, OH) saturated in liquid ant bait were used to deliver the toxicant (0.007% thiamethoxam) to Argentine ant colonies. To make 1 liter of bait, 500 ml of water was placed in a measuring container and 250 g of sucrose was added. When the sugar dissolved, water was added to bring it up to 1 liter to achieve 25% g/ml sucrose solution. To add the thiamethoxam, 1 ml of Optigard Flex (21.6% thiamethoxam; Syngenta Crop Protection Inc., Greensboro, NC) was diluted in 100 ml water, and 3.12 ml of the 1/100 dilution was then added to 1 liter of sucrose solution to achieve 0.007% thiamethoxam in 25% sugar water (based on 239.7 mg thiamethoxam/ml of Optigard Flex). Preliminary tests showed that the crystals reached maximum size and optimal saturation when 20 g dry crystals were mixed with 1 liter bait. Hence, 1 liter of 0.007% thiamethoxam in 25% sugar water to 20 g crystal ratio was used for each bait preparation, and the crystals were allowed at least 1 h to saturate.

Bait Aging Tests. Water-storing crystals were saturated with 25% sugar water containing 0.007% thiamethoxam as in Insecticide and Testing Substrates section. To determine water loss, the saturated crystals were placed in plastic weigh dishes (15 g per dish) and placed outdoors in the sun. In a second treatment, the bottom of the weigh dishes was lined with a thin layer of dry, rocky soil (10 g per dish) and 15 g of crystals were placed on top of the soil. This test was designed to determine whether water loss occurs faster when the crystals are placed directly on the soil as might happen under field conditions. The crystals and soil were weighed every hour for the first 8 h, then daily for 2 d. Six replicates were performed for each treatment. Climatic conditions (relative humidity and air temperature) were collected from the nearest National Oceanic and Atmospheric Administration (NOAA) reporting station.

Bait Efficacy Tests. Colony fragments consisting of 1,000 workers, 5 queens, and 50 mixed-instar larvae were placed inside 15- by 15- by 5-cm-high Fluon-coated plastic boxes and allowed to colonize a moist plaster nest (5 cm in diameter). The ants were provided with drinking water (ddH_2O) and allowed to acclimate to the nest for 24 h. No food was provided during the acclimation period. At the end of the acclimation period, the bait (5 g saturated crystals; 0.007% thiamethoxam) was introduced and fed ad libitum. Alternative food was not provided during the experiment to assess the efficacy of thiamethoxam in the absence of competing food sources. Five replications were performed and the assay was run for 6 d. On day 1, the attractiveness of the bait was estimated by monitoring worker recruitment to the bait every 30 min for 5 h. Subsequently, observations were made daily and consisted of 1) worker mortality, 2) queen mortality, and 3) brood condition. Brood condition was recorded using a visual assessment and rated according to the following scale: 5 = no change from the original or more, 4 = 70–90% brood present, 3 = 50% brood present, 2 = few brood present, and 1 = no brood present. Control tests (n = 5) consisted of monitoring worker recruitment and mortality in colonies provisioned with 5 g crystals saturated in untreated sugar water.

Efficacy of Aged Baits. Colony fragments consisting of 500 workers, 2 queens, and 25 mixed-instar larvae were placed inside 15- by 15- by 5-cm-high Fluon-coated plastic boxes and allowed to colonize a moist plaster nest (5 cm in diameter). The ants were provided with drinking water (ddH_2O) and allowed to acclimate to the nest for 24 h without food. At the end of the acclimation period, aged bait (5 g aged crystals; 0.007% thiamethoxam) was introduced and fed ad libitum. To age the bait, 5 g placements of bait (without soil) were aged outdoors (average relative humidity 31 ± 2% [range 22–49%] and average air temperature 83 ± 6°F [range 69–89°F]) for 1, 2, 4, 8, or 24 h. Five replications were performed for each time interval. On day 1, the attractiveness of the aged baits was estimated by monitoring worker recruitment to the baits every 30 min for 5 h. Subsequently, observations were made daily for 10 d and consisted of 1) worker mortality, 2) queen mortality, and 3) brood condition (as above).

Bait Uptake from Gelatin. The efficiency of thiamethoxam delivery to Argentine ants via gelatin cubes was examined under laboratory conditions. Gelatin and water crystals are similar in their properties (ability to retain water, consistency), and the goal for this objective was to compare the two and to determine whether either one was more advantageous for potential use in the field. Gelatin (Knox brand, unflavored, Kraft Foods Inc., Deerfield, IL) was dissolved in hot water containing 25% sucrose. An appropriate amount of Optigard Flex was added while the gelatin was still warm to achieve 0.007% concentration. The solution was chilled overnight to solidify. Preliminary tests showed that 10 g gelatin in 500 ml water gave the optimal balance between gelatin firmness and durability under field conditions (i.e., softer gelatin contains more water and is attractive for a longer period of time, but is less durable). Colony fragments consisting of 1,000 workers, 5 queens, and 50 mixed instar larvae were placed inside 15- by 15- by 5-cm-high Fluon-coated plastic boxes and allowed to colonize a moist plaster nest (5 cm in diameter). No food was provided during the acclimation period. At the end of the acclimation period, the bait (single gelatin cube; 2.5 cm on the side; 0.007% thiamethoxam) was introduced and fed ad libitum. Alternative food consisting of artificial Bhatkar diet was provided 24 h after the bait was introduced. Five replications were performed and the assay was run for 10 d. A control test consisting of gelatin containing no insecticide was performed as well (n = 5). On day 1, the attractiveness of the gelatin cube was estimated by monitoring worker recruitment to the bait every 30 min for 5 h. Subsequently, observations were made daily for 10 d and consisted of

1) worker mortality, 2) queen mortality, and 3) brood condition.

Horizontal Bait Transfer. This study examined the horizontal transfer of thiamethoxam from live treated donors to live untreated recipients. In addition, the effect of donor:recipient ratio (1:5, 1:10, 1:20) was tested. Recipient colonies consisting of 100 workers with 1 queen, 200 workers with 2 queens, or 400 workers with 4 queens were placed inside plastic boxes and allowed to colonize a moist plaster nest placed in the center of each box. The ants were provided with drinking water and allowed to acclimate to the nest for 24 h without food. To prepare the donors, a group of ≈1,000 workers was starved for 24 h and subsequently allowed to feed on a small pile (≈15 g) of crystals saturated in 0.007% thiamethoxam. Ants that fed to repletion (distended gasters) were gently removed from the feeding box by allowing them to walk onto a toothpick and immediately transferred to the recipient colony. Colonies consisting of 100 workers received 20 donors each (1:5 ratio), colonies consisting of 200 workers received 20 donors each (1:10 ratio), and colonies consisting of 400 workers received 20 donors each (1:20 ratio). The donors were introduced into the area outside the nest and allowed to freely interact with foragers present throughout the arena. Mortality in the treated donors and the untreated recipients was assessed daily for 10 d. Dead ants remained in the test boxes and were not removed. Each treatment was replicated five times.

Intracolony Bait Distribution. Water Storing Crystals were saturated in 25% sugar water containing 0.007% thiamethoxam and spiked with technical grade rabbit immunoglobin (IgG) protein (Sigma, St. Louis, MO) at a concentration of 0.5 mg IgG/ml bait. Colony fragments consisting of 500 workers, 2 queens, and 25 larvae were placed inside 15- by 15- by 5-cm-high Fluon-coated plastic boxes and allowed to colonize a moist plaster nest (5 cm in diameter). The ants were provided with drinking water (ddH_2O) and allowed to acclimate to the nest for 24 h. No food was provided during the acclimation period. At the end of the acclimation period, the bait (5 g; 0.007% thiamethoxam) was introduced and fed ad libitum. To estimate bait uptake and intracolony bait distribution, 15 workers were randomly sampled from outside of the nest for each replicate colony at 15 min, 1 h, 6 h, and 24 h after the bait was introduced. All individuals were frozen in individual tubes at −20°C and later analyzed by double antibody sandwich enzyme-linked immunosorbent assay using previously described methodology (Buczkowski and Bennett 2006, 2009). Five replicates were performed. The mean (±SE) percentage of samples scoring positive for rabbit immunoglobin protein were determined.

Statistical Analyses. All data analyses were performed using SAS 9.2 statistical software (SAS Institute 2008). For all assays, including bait aging tests, bait efficacy tests, efficacy of aged baits, and bait uptake from gelatin, statistical analysis consisted of analysis of variance (ANOVA) tests using the PROC GLM procedure on mean cumulative percent mortality. A CLASS statement was used to specify dependent classification variables used in the model. These variables included time (bait aging tests); caste, treatment, time (bait efficacy tests); age, caste, time, replicate (efficacy of aged baits); and caste, treatment, time, and replicate (bait uptake from gelatin). A *t*-test (TTEST) was used to compare mean worker mortality in assays on the efficacy of aged baits. The level of significance was set at $\alpha = 0.05$. Results are reported as mean ± SD.

Results

Bait Aging Tests. The bait crystals lost most of their weight in water within the first 8 h of outdoor exposure (Fig. 1A showing the difference between dry and fully saturated crystals). The duration of outdoor exposure had a highly significant effect on the amount of moisture lost by the crystals (ANOVA; df = 14; $F = 731.9$; $P < 0.0001$). During the first 8 h, the rate of water loss for crystals placed directly on dry soil versus crystals placed in a plastic dish without soil was comparable and the effect of treatment (soil vs no soil) was not statistically significant (ANOVA; df = 1; $F = 1.37$; $P = 0.243$). Little additional water loss was observed after the first 8 h. At 8 h, the percentage of water lost was similar for crystals with and without soil, 71 ± 0 versus 68 ± 2%, respectively (ANOVA; df = 5; $F = 1.20$; $P = 0.334$). At 48 h, the percentage of water lost by crystals exposed to soil was 75 ± 1% versus 74 ± 0% for crystals not exposed to soil (ANOVA; df = 5; $F = 0.72$; $P = 0.634$).

Bait Efficacy Tests. Bait crystals saturated in sugar water were highly attractive to Argentine ant workers. The average number of workers feeding on bait crystals containing thiamethoxam was 37 ± 8% (range, 19–69 workers) and 36 ± 6% (range, 15–82 workers) for crystals without thiamethoxam (ANOVA; df = 10; $F = 1.81$; $P = 0.421$). No significant difference was observed in the number of workers foraging on bait crystals with or without thiamethoxam, indicating that thiamethoxam is not repellent to Argentine ants at the 0.007% concentration.

Results also show that 0.007% thiamethoxam is highly effective against Argentine ants when delivered via bait crystals. The effects of all variables including treatment (thiamethoxam crystals vs control; ANOVA; df = 1; $F = 375.43$; $P < 0.0001$), caste (thiamethoxam crystals vs control; ANOVA; df = 2; $F = 8.23$; $P = 0.0004$), and time (thiamethoxam crystals vs control; ANOVA; df = 5; $F = 17.38$; $P < 0.0001$) were highly significant.

The majority of workers (>90%) died within 2 d of bait introduction, and complete worker mortality was observed within 3–6 d (Table 1). Relative to workers, mortality in the queens was somewhat slower, most likely owing to their larger body mass and lack of direct feeding on the baits. Queens generally started dying within 2 d, and complete mortality was observed within 6 d. Mortality in the brood was delayed as well and a gradual deterioration of brood condition was observed. Greater than 90% mortality was observed on day 4 and complete mortality was achieved by day 6.

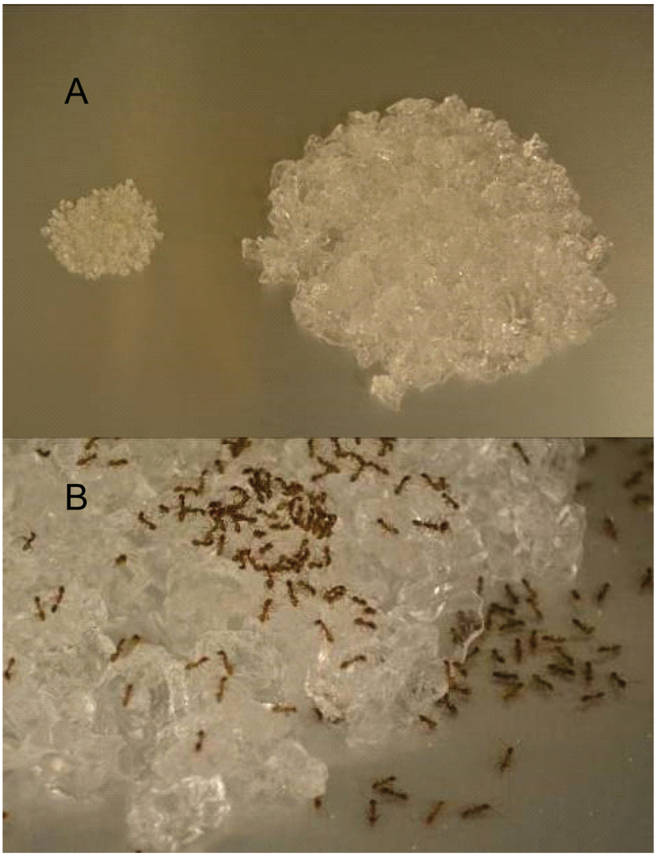

Fig. 1. Water-storing crystals: (A) 1 g of dry water-storing crystals (left) and 1 g of water-storing crystals fully saturated in water (right), (B) Argentine ants feeding on crystals saturated in 25% sucrose solution. (Online figure in color.)

The delay in queen and brood mortality may possibly reflect their dependence for sustenance and grooming by the workers. The brood became dry, shriveled up, and eventually moldy in the absence of worker care.

Efficacy of Aged Baits. Bait aging had a significant effect on bait acceptance as indicated by the number of workers feeding on the baits (ANOVA; df = 6; F = 125.62; $P < 0.0001$). As predicted, fresh bait was the most attractive. The average number of workers feeding on fresh bait was 37 ± 19. Baits aged for 1, 2, or 4 h were substantially less attractive relative to fresh bait, with 25 ± 20 workers feeding on 1-h-old bait (32% reduction relative to fresh bait), 20 ± 15 feeding on 2-h-old bait (46% reduction), and 21 ± 19 feeding on 4-h-old bait (43% reduction). Baits aged for 8 and 24 h were significantly less attractive compared with fresh baits or those aged for 1, 2, or 4 h. The number of workers feeding on the baits declined to 13 ± 11 workers on 8-h-old baits (65% reduction) and 11 ± 10 workers on 24-h-old baits (70% reduction).

With regard to bait efficacy, a significant relationship between bait age and bait efficacy was observed depending on the aging interval (Fig. 3; ANOVA; df = 6; F = 255.06; $P < 0.0001$). There was no significant difference in worker mortality between fresh baits or those aged for 1 h (t-test; df = 9; t = 2.62; P = 0.213)

Table 1. Cumulative percent mortality (± SD) in Argentine ant workers, queens, and brood exposed to fresh bait crystals containing 0.006% thiamethoxam

Caste	Treatment	Time (d)					
		1	2	3	4	5	6
Workers	Thiamethoxam	24 ± 6a	94 ± 2a	99 ± 1a	100 ± 0a	100 ± 0a	100 ± 0a
Workers	Control	1 ± 0b	2 ± 0b	2 ± 1b	3 ± 1b	3 ± 1b	4 ± 1b
Queens	Thiamethoxam	0 ± 0a	40 ± 42a	70 ± 45a	80 ± 27a	80 ± 27a	100 ± 0a
Queens	Control	0 ± 0a	0 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b
Brood	Thiamethoxam	0 ± 0a	9 ± 8a	36 ± 19a	94 ± 5a	98 ± 4a	100 ± 0a
Brood	Control	0 ± 0a	0 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b

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

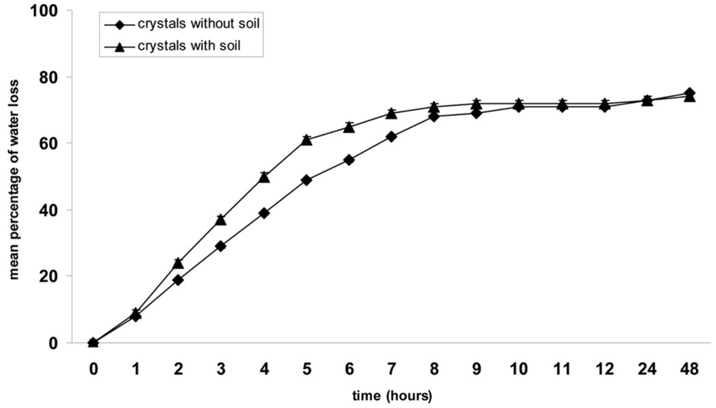

Fig. 2. Mean (± SD) percentage of water loss experienced by water-storing crystals exposed to outdoor conditions for 48 h.

or 2 h (*t*-test; df = 9; *t* = 2.26; *P* = 0.186). Fresh baits and those aged for 1 or 2 h gave 95% mortality in the first 2 d and 100% mortality within 7 d (Fig. 2A). The rate of mortality for bait crystals aged for 4 h was significantly slower relative to fresh bait (*t*-test; df = 9; *t* = 1.83; *P* = 0.002), and worker mortality did not exceed 94 ± 4% at 10 d after treatment. Relative to fresh bait, baits aged for 8 or 24 h killed substantially fewer workers, with worker mortality around 50–60% at 10 d after treatment. The 8 and 24 h baits showed similar patterns of worker mortality, corroborating the results of the bait aging tests, which showed that most of the water loss occurs in the first 8 h, with no significant loss after that. Regarding queen mortality, a gradual decrease in queen mortality was observed as bait age increased (Fig. 3B). In general, fresh baits and those aged for 1–4 h retained their efficacy and caused >80% mortality. In tests with 8-h-old baits, all but 1 of the 10 queens in the five replicate colonies survived, and no mortality was observed with 24-h-old baits. Similarly, brood condition was affected to a varying degree depending on bait age (Fig. 3C). Within the 10-d test period, baits aged for 1–2 h resulted in complete brood elimination, baits aged for 4 h caused almost complete brood elimination (mean score of 2 ± 1), and baits aged for 8 or 24 h had little to no effect on brood mortality (mean scores of 4 ± 0 and 5 ± 0, respectively).

Bait Uptake from Gelatin. Gelatin cubes were highly effective in delivering thiamethoxam to Argentine ants (Table 2). Gelatin cubes containing thiamethoxam were significantly more effective than gelatin cubes containing no insecticide (control tests; ANOVA; df = 1; *F* = 188.52; *P* < 0.0001). Gelatin cubes containing thiamethoxam were highly effective against workers, but mortality in queens and brood was somewhat delayed with caste a highly significant factor (ANOVA; df = 2; *F* = 16.42; *P* < 0.0001). The majority of workers (89 ± 7%) died within 2 d of bait introduction, with complete worker mortality observed within 3 d. Mortality in the queens was somewhat slower and the queens generally started dying within 2–3 d. Complete queen mortality was achieved within 5 d. Likewise, mortality in the brood was delayed and reached 98 ± 3% on day 6.

Thiamethoxam delivery from gelatin cubes was highly comparable with that observed for hydrogel crystals. The average number of workers feeding on the gelatin bait over the 5-h period was 38 ± 5, not statistically different from 37 ± 8, the number of workers that fed on bait crystals (*t*-test; df = 10; *t* = 1.81; *P* = 0.375). The 5-d results for worker, queen, and brood mortality following feeding on gelatin cubes were largely similar to those obtained for hydrogel crystals (Tables 1 and 2), suggesting similar efficiency in thiamethoxam delivery. This suggests that both gelatin cubes and hydrogel crystals are effective in delivering the bait to Argentine ants.

Horizontal Bait Transfer. As expected, the highest level of recipient worker mortality (expressed as percent mortality) was observed in the highest donor: recipient ratio of 1:5 (Table 3) When 20 bait-fed donor workers were introduced into a colony of 100 untreated recipient workers, the resulting mortality in the recipient workers reached 40% 10 d. The 1:10 and 1:20 ratios resulted in significantly lower recipient mortality, around 15% (Table 3). No queens died in any of the transfer tests, suggesting that multiple feedings from multiple donors may be necessary to kill the queens. Overall, the results of the transfer tests confirm the horizontal transfer of thiamethoxam and demonstrate the role of trophallaxis in the distribution and efficacy of thiamethoxam.

Intracolony Bait Distribution. Bait distribution in colonies was examined through IgG protein incorporated into the bait crystals. Bait distribution was fairly rapid, with 79 ± 13% of workers testing positive at 15 min after bait introduction. The percentage of workers testing positive increased to 93 ± 5% at 1 h, then 100 ± 0% at 6 h and 24 h.

Discussion

Argentine ants have reached pest status in urban, agricultural, and natural environments (Silverman and Brightwell 2008) where they typically associate

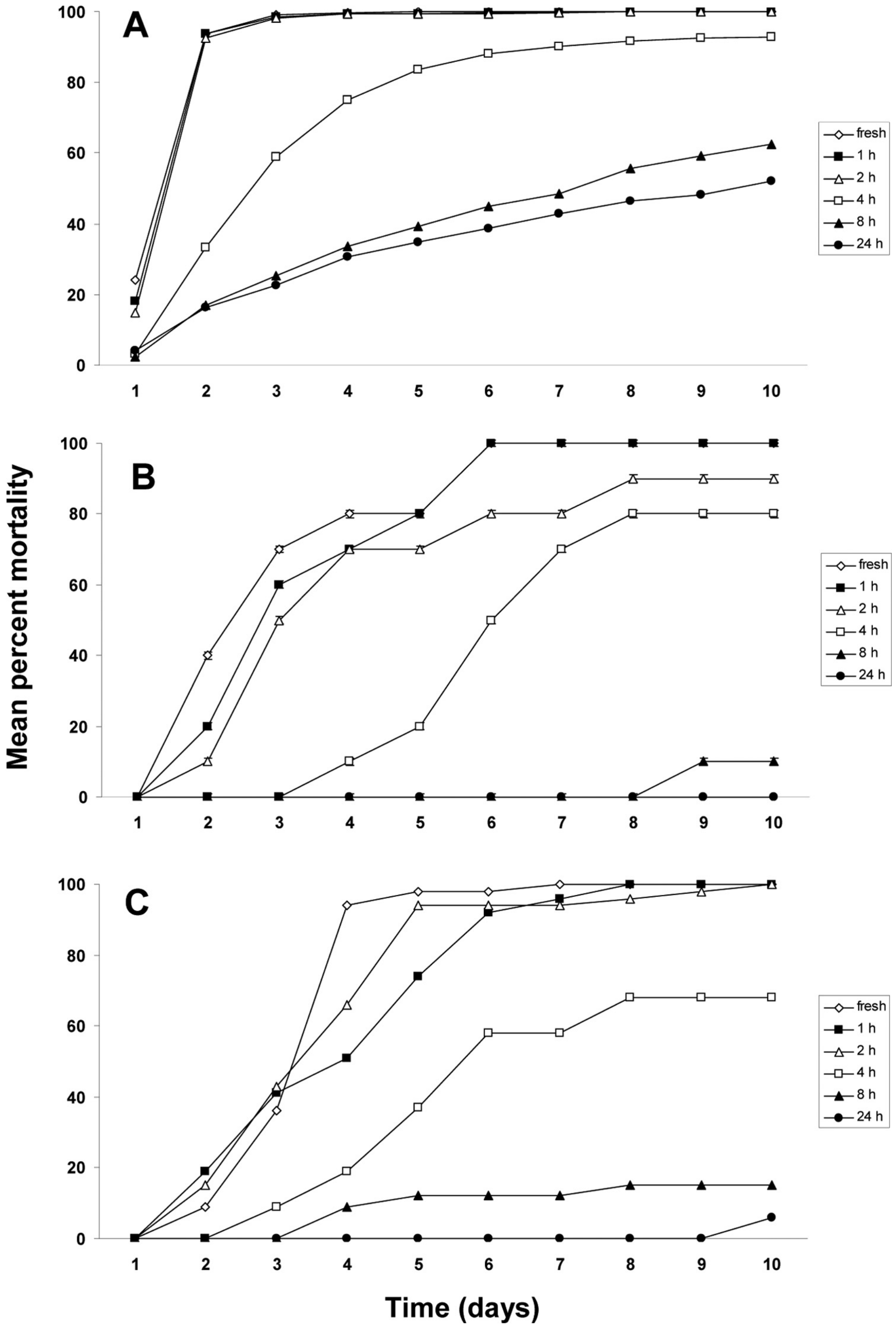

Fig. 3. Mean percent mortality in Argentine ants exposed to fresh and aged bait crystals containing 0.006% thiamethoxam (A) worker mortality, (B) queen mortality, and (C) brood mortality. Standard deviations omitted for clarity.

with honeydew-producing hemipterans and collect their honeydew. Because of their natural affinity for liquid foods rich in carbohydrates (e.g., honeydew, nectar, tree sap), Argentine ant management is best achieved with liquid sugar baits containing an active toxicant (e.g., Nelson and Daane 2007, Daane et al. 2008, Brightwell and Silverman 2009). However, liquid bait use has been limited owing to the lack of bait

Table 2. Cumulative percent mortality (± SD) in Argentine ant workers, queens, and brood exposed to fresh gelatin cubes containing 0.006% thiamethoxam

Caste	Treatment	Time (d)					
		1	2	3	4	5	6
Workers	Thiamethoxam	16 ± 6a	89 ± 7a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a
Workers	Control	1 ± 1b	2 ± 1b	3 ± 1b	3 ± 1b	3 ± 1b	4 ± 1b
Queens	Thiamethoxam	0 ± 0a	20 ± 27a	80 ± 27a	90 ± 22a	100 ± 0a	100 ± 0a
Queens	Control	0 ± 0a	0 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b
Brood	Thiamethoxam	0 ± 0a	0 ± 0a	23 ± 25a	36 ± 19a	78 ± 26a	98 ± 3a
Brood	Control	0 ± 0a	0 ± 0a	0 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b

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

dispensers that are effective, inexpensive, and easy to service especially when used on a commercial scale. The current study evaluated the potential of superabsorbent acrylamide polymers (known as water-storing crystals or hydrogels) to deliver liquid baits to laboratory colonies of Argentine ants.

Results show that under laboratory conditions water-storing crystals saturated in 25% sucrose solution containing 0.007% thiamethoxam are highly attractive and highly effective against Argentine ants and require ≈6 d to kill all castes and life stages in Argentine ants. Results of bait aging tests demonstrated that the crystals gradually lose moisture over time, but remain attractive and effective for ≈2–4 h. The crystals lost 70% of their mass in the first 8 h and little additional water loss was observed after the first 8 h. Results show that placing the crystals directly on dry soil does not significantly contribute to water loss. This suggests that under field situations the crystals may be placed directly on the ground and should not require any special dispensers or containers to protect them from desiccation. This is especially important if the crystals were to be used in ant eradication efforts over large areas such as natural ecosystems or commercial farms. Under such conditions the saturate crystals could be applied directly to the ground using existing farm equipment such as granular fertilizer spreaders or seed dispensers. However, the results presented here are specific for the climatic conditions recorded during the test, with average relative humidity at 31 ± 2% (range, 22–49%) and average air temperature at 83 ± 6°F (range, 69–89°F). Future tests should examine water loss from crystals exposed to a wider range of climatic conditions, especially arid climates where the crystals may potentially be used.

Bait attractiveness decreased significantly with increasing outdoor exposure, and baits aged for 1, 2, or 4 h were substantially less attractive relative to fresh bait. The results show that the ants have a rather narrow window to discover and consume the baits. Crystals exposed to outdoor conditions for >4 h lost a substantial proportion of water, which limited the amount of sugar water (and thus thiamethoxam) available to the ants. A significant negative relationship between bait age and bait efficacy was observed, depending on the aging interval, and a similar result was observed by Boser et al. 2014. However, even crystals that lost >70% of moisture (those aged for 24 h) retained some attractiveness and resulted in ≈50% worker mortality during a 10-d exposure. The results suggest that under field conditions the crystals should be applied close to foraging trails and nests to minimize time necessary for bait discovery and maximize time available for feeding. Furthermore, the crystals should be applied during cooler parts of the day to minimize the rate of water loss. It remains to be determined whether dried out crystals can regain efficacy if they become resaturated following irrigation or rainfall. Additional tests should determine whether dried out crystals remain toxic to ants that walk over the crystals (contact activity) and whether saturated crystals leach the active ingredient into the soil, which could then act as a contact insecticide.

The results of the transfer tests confirm the horizontal transfer of thiamethoxam and demonstrate the role of trophallaxis in the distribution of thiamethoxam. Thiamethoxam transfer was highly efficient in the workers, but no mortality was observed in the queens. This suggests that multiple feedings may be necessary to kill the queens, which typically do not forage on their own and rely on workers for nutrition. As with other insecticides, primary and secondary mortality are largely determined by the insecticide's speed of action and concentration. Higher doses typically cause higher mortality in individuals that feed directly (primary mortality), but have reduced potential for horizontal transfer (secondary mortality) because the donors die before they are able to share

Table 3. Cumulative percent mortality (± SD) in untreated recipient Argentine ant workers exposed to bait-fed donors workers

Caste	Ratio	Time (d)									
		1	2	3	4	5	6	7	8	9	10
Workers	1:5	2 ± 4	19 ± 14	23 ± 17	25 ± 17	29 ± 16	30 ± 15	35 ± 14	36 ± 13	38 ± 14	40 ± 13
Workers	1:10	0 ± 0	6 ± 4	6 ± 4	8 ± 6	9 ± 7	10 ± 8	13 ± 9	14 ± 9	15 ± 10	17 ± 10
Workers	1:20	3 ± 2	7 ± 4	7 ± 5	9 ± 5	10 ± 5	11 ± 5	13 ± 6	14 ± 6	16 ± 7	18 ± 5

No mortality in the queens was observed.

the insecticide. Lower doses typically have more potential for transfer, but may ultimately result in lower mortality if the insecticide becomes too dilute, as it is shared via trophallaxis. Future tests should pinpoint the amount of thiamethoxam necessary to induce mortality on the individual level and screen a wider range of doses to discover an optimal balance between thiamethoxam concentration and the resulting primary and secondary mortality.

Results of protein marking tests that incorporated IgG protein into the bait crystals demonstrate that the distribution of sugar water containing 0.007% thiamethoxam in colony fragments consisting of 500 workers is rapid and complete with >90% of the workers testing positive in 1 h and 100% testing positive in 6 h. This result is similar to previous studies that used protein marking and investigated the flow of 25% sucrose solution in laboratory and field colonies of odorous house ants (Buczkowski and Bennett 2006, Buczkowski and VanWeelden 2010). In the current study, the fast spread of sucrose (and therefore thiamethoxam) was likely owing to the relatively small colony size (500 workers), relatively proximity to the bait, and lack of alternative food sources. However, some workers may have tested positive after only touching the bait, and it is not known how many workers actually ingested the bait or how much bait was ingested by the individual workers.

Further tests using larger laboratory colonies or natural field colonies should be carried out to determine the extent of bait distribution under more realistic field conditions and to gain a better understanding of how social behavior and colony organization in unicolonial ants affects toxicant transfer. Field populations of Argentine ants are unicolonial and are composed of multiple interconnected nests containing millions of workers and thousands of queens (e.g., Markin 1970, Suarez et al. 1999). Nest connectivity within such colonies and the degree of communication and food sharing among the individual nests are not well understood, although previous tests show that communication and worker exchange in unicolonial populations of Argentine ants (Heller et al. 2008) and odorous house ants (Buczkowski and Bennett 2006) is somewhat limited. Specifically, the large populations do not function ecologically as single, large supercolonies but as smaller distinct colonies consisting of groups of interacting nests. A field study by Buczkowski and Bennett (2006) used protein marking to investigate the foraging pattern and foraging range in supercolonies of odorous house ants, *Tapinoma sessile* (Say), and showed that foraging is highly localized. The ants engage in dispersed central place foraging whereby members of individual nests use local food sources with little or no exchange of individuals or food among neighboring nests. The ants exhibit high foraging site fidelity, travel along well-established trails, forage on a local scale, and consistently deliver harvested resources to specific nests. A priority for future studies should be to investigate the movement of thiamethoxam from the bait crystals in field populations of Argentine ants. The main goal should be to determine the optimal density of bait placements necessary to achieve efficient bait distribution across the whole population.

In summary, the results of the current study demonstrate that bait crystals saturated in 25% sugar water containing 0.007% thiamethoxam are highly effective against laboratory colonies of Argentine ants and have the potential for development as an effective ant management tool. Future studies should aim to test the crystals under field conditions to determine their attractiveness and efficacy on large unicolonial populations that may have access to alternative food sources. Studies that evaluate the crystals across different ecosystems should be especially valuable to determine their efficacy and utility in urban, natural, and agricultural situations. In addition, studies that test different delivery methods (e.g., scattered vs clumped applications), different active ingredients and feeding attractants, and a wider range of pest species should be of special value.

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