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USE OF AN ALGINATE HYDROGEL TO DELIVER AQUEOUS BAIT TO MANAGE AN INVASIVE ANT PEST IN RESIDENTIAL SETTINGS

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Abstract Insecticide sprays used for ant control generally cause environmental contamination. Liquid bait is effective, but requires bait stations to dispense the toxicant. In this study, we develop a natural alginate hydrogel to deliver liquid bait, without the use of bait stations. Hydrogel beads conditioned in a sucrose solution with 0.0001% thiamethoxam provided complete control of all castes of Argentine ant *Linepithema humile* (Mayr) colonies, by 5 days post-treatment in the laboratory trial, and provided a 79% reduction in ant activity after 8 weeks in the field trial. This work demonstrates the potential of alginate hydrogel as an effective tool to deliver liquid bait in controlling Argentine ants.

Key words Argentine ant, ant control, biodegradable hydrogel, *Linepithema humile*, liquid bait, thiamethoxam

INTRODUCTION

The Argentine ant, *Linepithema humile* (Mayr), is among the most successful pest ant species in urban (Knight and Rust, 1990), agricultural (Daane et al., 2008), and natural environments (Boser et al., 2014). Sucrose liquid bait has been demonstrated to effectively control Argentine ants (Rust et al., 2004). However, they require that bait stations be implemented, which are costly and require frequent maintenance (Nelson and Daane, 2007; Rust et al., 2015). The sucrose liquid baits in the bait station also tends to ferment under warm environmental conditions, consequently compromising its attractiveness (Cooper et al., 2008; Buczkowski et al., 2014a).

To date, a synthetic hydrogel made of polyacrylamide was tested to deliver sucrose liquid baits to Argentine ants, without the use of bait stations (Boser et al., 2014; Buczkowski et al., 2014a; Buczkowski et al., 2014b; Rust et al., 2015). The use of hydrogels makes it possible to apply liquid bait directly on the ground, near areas where ants are foraging and nesting. Polyacrylamide hydrogel, however, degrades into acrylamide, a chemical listed by the World Health Organization as being toxic (World Health Organization, 1985). We report the effectiveness of alginate, a natural polymer derived from seaweed, to deliver sucrose liquid bait to Argentine ants.

MATERIALS AND METHODS

Ants

Linepithema humile colonies were collected from a citrus grove located at the University of California, Riverside, CA, USA, and were maintained in the laboratory.

Preparation of Alginate Hydrogels

A 1% sodium alginate (Na-Alg) solution was prepared by dissolving 1 g of Na-Alg (Sigma Aldrich, St. Louis, MO, USA) in 100 ml of deionized water. The Na-Alg solution was dispensed dropwise into the crosslinker, 0.5% calcium chloride ($CaCl_2$) using a 5 ml syringe. The resulting hydrogel beads were filtered out after 5 minutes, and then rinsed with deionized water. Each bead was conditioned in 100ml of the 25% sucrose solution containing 0.0001% (wt:vol) analytical grade thiamethoxam (Thiamethoxam PESTANAL®, Sigma Aldrich, St. Louis, MO, USA), which resulted in the complete hydration of the hydrogel beads. The concentrations of Na-Alg (1%) and $CaCl_2$ solutions (0.5%), and crosslinking time (5 minutes) was optimized to produce hydrogel beads with preferred rigidity and maximum hydration in a 25% sucrose solution (Tay et al., unpublished data).

Laboratory Small Colony Test

A total of 300 workers, 2 queens, and 0.1g of brood were separated from the main colony. Each colony was then reared in a polyethylene container (33 x 19 x 10 cm), the sides and inner surface of which were coated with a thin film of teflon (polytetrafluoroethylene suspension; BioQuip, Rancho Dominguez, CA, USA). A petri dish (10 cm in diameter and 1.5 cm in height) with 4 small entry holes containing folded corrugated paper (14 x 6 cm), served as harborage. On a weekly basis, the ants were provided with water, a 25% sucrose solution, freshly-killed cockroaches, and canned tuna fish. The colonies were acclimatized for a 7-day period, and were then starved for 3 days, prior to baiting. At the completion of the starvation period, 3 newly prepared hydrogel beads were introduced in each colony box. The treated colonies were provided with hydrogels conditioned in a 25% sucrose solution with 0.0001% analytical grade thiamethoxam for 24 hours. Control colonies were provided with hydrogel conditioned in a 25% sucrose solution without any toxicants. At 24 hours post-treatment, the colonies were re-assigned to their original diet. The number of live queens and workers were counted from photographs, and the weight of the brood was measured at 1, 3, and 5 days post-treatment. The experiment was replicated 5 times. The percent reductions in the number of workers, queens, and brood between the treated and untreated colonies were arcsine square-root transformed and analyzed using paired *t*-test (SPSS Inc, 2002).

Field Efficacy Test

Droplets of 1% Na-Alg solution were produced using a 100-nozzle shower head (AKDY AZ-6021 8-inch bathroom chrome shower head, CA, USA), and were collected in a plastic container (381 x 292 x 152 mm) with a 0.5% $CaCl_2$ cross linker solution. The resulting alginate hydrogel beads prepared from a 5 L of Na-Alg solution were filtered out and conditioned in a 5 L of 50% sucrose solution with 0.0002% of thiamethoxam for a 24-hour period. It was assumed that concentrations of thiamethoxam and sucrose solution inside and outside of the hydrogel beads reached equilibrium after the 24-hour conditioning period, which produced alginate hydrogel baits containing about 25% sucrose solution with ~ 0.0001% of thiamethoxam. The hydrogel baits were then filtered out from the liquid bait. Their efficacy was tested at 5 residential houses in Riverside, CA, USA. Each experimental site was treated with ~ 1 kg of hydrogel bait in an application rate of 10 grams m^{-2}, applied in ~ 20 piles (~ 50 grams per pile), within 5 m from the structure. Estimations of foraging activity levels of Argentine ants, before and after treatment, were based on the total amount of sucrose solution consumed by ants over a 24-hour period. On each monitoring date, 20 monitoring vials (15 ml Falcon plastic vial, BD bioscience, San Jose, CA, USA), each containing 12 ml of 25% sucrose solution, were placed at 10 different spots around each structure. At each spot, a set of 2 vials was placed on the ground in the notch of 2 Lincoln Logs™, and was covered with a flower pot (15.5 cm in diameter and 11.5 cm in height). The amount of sucrose solution consumed by the ants was determined by measuring the difference between the initial and finial weights of the vials over 24 hours, and then correcting for evaporation (weight loss from another set of monitoring vials which ants could not access). The number of ant visits to each vial was estimated by dividing the amount of sucrose solution an Argentine ant can consume (0.3 mg) per visit (Reierson et al., 1998). Field sites were monitored on day 1 pre-treatment, and weeks 1, 2, and 4 post-treatment. The second treatment with hydrogel bait was made in the same manner, after the monitoring at week 4, and sites were further monitored at weeks 5, 6, and 8 post-treatment (calculated from the dates of the first treatment deployment). The average number of ant visits recorded at each site for all monitoring dates was square-root transformed. The number of ant visits at each post-treatment monitoring date was compared with the pre-treatment level with paired *t*-tests at the 0.05 level of significance (SPSS Inc, 2002).

Table 1. Percentage reduction of worker, queen, and brood ants (mean ± SEM) in laboratory study after baiting with alginate hydrogel baits containing 0.0001% thiamethoxam.

Treatment	Caste	Time (Day) [a]		
		1	3	5
Control	Worker	9.87 ± 3.07a	12.13 ± 4.23a	13.93 ± 2.12a
	Queen	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
	Brood	10.00 ± 6.32a	-2.00 ± 12.41a	16.00 ± 8.72a
0.0001% thiame-thoxam	Worker	22.93 ± 5.55a	89.07 ± 1.24b	100.00 ± 0.00b
	Queen	0.00 ± 0.00a	40.00 ± 10.00b	100.00 ± 0.00b
	Brood	22.00 ± 5.83b	78.00 ± 7.35b	100.00 ± 0.00b

[a] Means with the same letter for each caste within a column are not significantly different at $\alpha = 0.05$ (data were arcsine square-root transformed; paired *t*-test).

Table 2. Pre- and post-treatment average ant visits at 5 sites treated with hydrogel baits containing 0.0001% thiamethoxam.

Site	Pre-treatment average ant visits	Post-treatment average ant visits [a]					
		1 week	2 weeks	4 weeks	5 weeks	6 weeks	8 weeks
1	54,199	26,166	32,707	18,474	15,058	22,236	13,261
2	70,197	24,508	30,217	8,284	13,871	9,048	6,229
3	30,517	26,925	28,133	23,460	18,316	26,294	11,033
4	46,826	32,490	29,689	8,029	5,887	15,180	5,672
5	68,741	35,272	30,304	15,077	14,253	17,474	15,314
Mean	54,096	29,072*	30,210*	14,665*	13,477*	18,046*	10,302*

[a] A second bait treatment was made at all sites between week 4 and 5

* Significant at $\alpha = 0.05$ compared with corresponding pre-treatment average ant visits (data were square-root transformed; paired *t*-test)

RESULTS

In the laboratory study, significant differences in the percent brood reduction were recorded in the treated colonies, when compared to the control at day 1 post-treatment. The hydrogel baits containing 0.0001% thiamethoxam provided 100% control for workers, queens, and brood by day 5 post-treatment (Table 1). In the field study, an average of 42, 40, 68, 72, 61, and 79% reduction in ant visits were recorded for week 1, 2, 4, 5, 6, and 8, respectively, when compared to the corresponding pre-treatment data. Average ant visits to monitoring vials in all post-treatment monitoring dates were significantly lower than their respective pre-treatment estimates, across 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 2).

DISCUSSION

Alginates are polysaccharides that are widely distributed in nature. They consist of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) (Murata et al., 2004). The calcium alginate hydrogel utilized in this study was created by the ionical interaction between Na-Alg and calcium ions (Saarai et al., 2011). Alginate hydrogel containing low concentration of thiamethoxam (0.0001%) shows high efficacy in the laboratory and field trials. The alginate hydrogel bait immediately provided a 42% ant reduction, on average, by week 1 post-treatment in the field trial. After a second treatment between weeks 4 and 5, the alginate hydrogel baiting maintained 61 – 79% ant reductions, on average, until the completion of the experiment. A recent similar laboratory small colony test demonstrates that alginate hydrogel conditioned in a 25% sucrose solution with 0.5% boric acid, a lower risk insecticide, achieved complete mortality in all castes of Argentine ant by day 14 post-treatment (Tay, unpublished data). An in-depth field study is currently in progress to evaluate its efficacy in the field. The use of alginate hydrogel baits allows effective ant management possible with minimal amounts of insecticide. It is also environmentally friendly as it does not leave any toxic on degradation.

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