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ORIGINAL PAPER

Biodegradable alginate hydrogel bait delivery system effectively controls high-density populations of Argentine ant in commercial citrus

Kelsey A. McCalla[1,6] · **Jia-Wei Tay**[1,5] · **Ashok Mulchandani**[2,3] · **Dong-Hwan Choe**[1] · **Mark S. Hoddle**[1,4]

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Abstract

The Argentine ant, *Linepithema humile* (Mayr), poses a significant economic threat to citrus production in southern California. Broad-spectrum insecticide sprays provide poor ant control and present a hazard to human and environmental health. Liquid sucrose bait infused with a low concentration of insecticide is an effective alternative treatment but current approaches require considerable economic investment in plastic dispensers and continual maintenance. To produce a baiting product for control of *L. humile* suitable for large-scale application, a biodegradable, broadcastable alginate hydrogel for delivery of aqueous low-dose thiamethoxam sucrose bait was developed and evaluated in replicated field trials in commercial citrus groves. Ant activity was significantly reduced in hydrogel-treated trees. Peak *L. humile* suppression was achieved 48 h following the final hydrogel disbursement, with an estimated 91% reduction in activity from baseline estimates and a 17-fold lower activity in treated trees in comparison with untreated trees. Significant residual activity of the hydrogel treatments was recorded, with a nearly 70% reduction from pre-treatment levels persisting at least 3 weeks after the last application. We conclude that alginate hydrogels can provide excellent control of *L. humile* while deploying 99.99% less insecticide into orchards than commercial barrier spray treatments.

Keywords Argentine ant · Commercial citrus · Ant control · Bait · Biodegradable hydrogel · Thiamethoxam

Key message

- Current chemical management options for Argentine ant in California citrus production are insufficient.
- Broadcast applications of biodegradable alginate hydrogels loaded with liquid bait were evaluated for control of Argentine ant in commercial citrus groves.
- Hydrogel treatments demonstrated high field efficacy using negligible quantities of insecticide at a cost substantially lower than commercial bait-and-dispenser programs.
- Alginate hydrogels have strong potential for large-scale commercial agricultural use where control of Argentine ant or other sugar-feeding ants is needed.

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✉ Kelsey A. McCalla
kelseyamccalla@gmail.com

[1] Department of Entomology, University of California Riverside, 900 University Ave., Riverside, CA 92521, USA

[2] Department of Chemical Engineering, University of California Riverside, 900 University Ave., Riverside, CA 92521, USA

[3] Materials Science and Engineering Program, University of California Riverside, 900 University Ave., Riverside, CA 92521, USA

[4] Center for Invasive Species Research, University of California Riverside, 900 University Ave., Riverside, CA 92521, USA

[5] Present Address: Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Maile Way, Gilmore Hall 310, Honolulu, HI 96822, USA

[6] Present Address: Department of Environmental Science, Policy, and Management, University of California Berkeley, Berkeley, CA 94720, USA

Introduction

The invasive Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), is a highly destructive pest of natural and managed systems worldwide (Vega and Rust 2001; Silverman and Brightwell 2008). Although primarily recognized as an urban structural pest, large, persistent infestations of *L. humile* are also sustained in agroecosystems. Citrus orchards in southern California provide optimal proliferative conditions for *L. humile*: a mild Mediterranean climate, year-round moisture from irrigation, and abundant honeydew-producing hemipteran mutualists (Vega and Rust 2001). Individual citrus trees have been recorded to receive several hundreds of thousands of visits from *L. humile* foragers in a single 24-h period (Markin 1970; Milosavljević et al. 2017; Schall and Hoddle 2017). Workers may obstruct irrigation piping, invade commercial beehives, disrupt native pollinators, and impede biological control of the sap-feeding pests they tend, resulting in pest outbreaks and concomitant plant damages (Buckley 1987; Vega and Rust 2001; Silverman and Brightwell 2008; Hanna et al. 2015). Previous studies have demonstrated the importance of *L. humile* control for management of ant-tended pests in citrus (e.g., Asian citrus psyllid, *Diaphorina citri* Kuwayama) which threaten the profitability of California's $7.1 billion-per-year citrus industry (Tena et al. 2013; Milosavljević et al. 2017; Schall and Hoddle 2017; Babcock 2018). However, tools with which to implement an effective and economically feasible population-level suppression program for *L. humile* in commercial citrus are limited.

Conventional treatment for *L. humile* typically consists of a barrier spray application of insecticide around the base of crop plants to limit access and kill workers upon contact with residue (Rust et al. 2004; Tollerup et al. 2004; Silverman and Brightwell 2008). In California citrus production, formulations approved for *L. humile* control include an emulsifiable concentrate (i.e., Lorsban-4E and Lorsban Advanced), wet powder (i.e., Lorsban-75WE), and granular application (i.e., Lorsban-15G) of chlorpyrifos. Chlorpyrifos residues kill or repel ants foraging on the soil surface but have little impact on the subterranean colony where the queens, brood, and majority of workers reside (Knight and Rust 1990b; Vega and Rust 2001). In addition, residues are highly susceptible to environmental conditions (i.e., degrade in high heat, are diluted and dispersed with irrigation water or rain, and may be circumvented through alternate canopy routes) (Rust et al. 1996). The limited residual activity of chlorpyrifos treatments in combination with high availability of replacement nestmates and immigration of foragers from untreated areas often necessitates monthly reapplication (Knight and Rust 1990a; Rust et al. 1996; Daane et al. 2008). Additionally, as a potent broad-spectrum insecticide, chlorpyrifos can cause considerable mortality of natural enemies needed to manage infestations of honeydew-producing hemipterans and other pests (Bellows and Morse 1988; Thomson and Hoffmann 2006). Plant damage associated with *L. humile* infestation is primarily indirect, incurred through disruption of biological control and concomitant outbreaks of hemipteran mutualists (Schall and Hoddle 2017; Schall et al. 2018). Consequently, ant control confers little net benefit if populations of natural enemies are extirpated in the process.

Despite significant drawbacks, chlorpyrifos barrier treatments are the prevailing method of ant control employed in commercial citrus. They are easily applied, provide immediate albeit temporary suppression of foraging ants, and few other cost-effective treatment options are available (Buczkowski et al. 2014b; Welzel and Choe 2016). However, chlorpyrifos was banned in California in early 2019 due to its potential to cause developmental neurotoxicological effects in children at low-dose levels (Environmental Protection Agency 2016; California Department of Pesticide Regulation 2019a, b). Usage is mandated to cease by 2021, and replacement ant control products will be needed.

Baiting programs are excellent alternatives to conventional barrier sprays. They exploit the social behavior of ants to provide long-term, colony-wide suppression while preserving natural enemies and minimizing environmental contamination (Cooper et al. 2008; Milosavljević et al. 2017; Schall and Hoddle 2017). Unlike broad-spectrum insecticide sprays which kill upon contact, the delayed toxicity of baiting treatments provides sufficient time for mass recruitment of workers to bait sources and trophallactic dissemination of the toxicant among colony members (Rust et al. 2004; Silverman and Brightwell 2008). The result is nest-wide collapse. In order to be effective, baits must be formulated to be more attractive than nearby food resources, non-repellent, easily transferrable to nestmates, and efficacious under field conditions (i.e., minimal photodegradation in sunlight or palatability loss following evaporation) (Silverman and Brightwell 2008).

Baits are typically comprised of a low-concentration toxicant and a phagostimulant (e.g., sugar, oil, or protein) and can be delivered in a granular, gel, or liquid formulation. Aqueous sucrose compositions are ideal for control of sugar-feeding ants such as *L. humile* as they are inexpensive to produce and resemble honeydew, a preferred natural food source collected year-round (Markin 1970; Silverman and Brightwell 2008; Abril et al. 2014). A sucrose concentration of 20–25% maximizes *L. humile* forager recruitment to liquid baits, toxicant intake and transfer, and colony mortality (Silverman and Roulston 2001; Sola and Josens 2016). Commonly evaluated toxicants used in liquid bait formulations for control of *L. humile* include hydramethylnon, boric acid, fipronil, thiamethoxam, spinosad, and imidacloprid (Klotz et al. 2003; Tollerup et al.

2004; Greenberg et al. 2006; Cooper et al. 2008; Silverman and Brightwell 2008). Thiamethoxam-based baits have been reported to reliably control *L. humile* populations under both laboratory and field conditions across a wide range of concentrations (Cooper et al. 2008; Buczkowski et al. 2014a, b; Rust et al. 2015; Schall and Hoddle 2017).

In addition to providing colony-level control of *L. humile*, liquid baiting programs minimize off-target effects and environmental contamination. Narrow bait dispenser openings physically occlude larger arthropods, and containment of bait in a reservoir reduces runoff (Cooper et al. 2008). Furthermore, experimental baiting treatments deploy 15,000 to 42,000 times less toxicant into the environment than conventional spray applications of chlorpyrifos (Table 1). However, the standard liquid bait-and-dispenser design has limited feasibility for commercial agricultural production. Dispensers must be deployed in high densities to control *L. humile* infestations (Nelson and Daane 2014) and bait deployed for extended periods of time is susceptible to fermentation and evaporative loss, which may render it unpalatable or ineffective (Silverman and Brightwell 2008; Buczkowski et al. 2014a, b). Consequently, bait stations require continual servicing (e.g., refilling, replacement, cleaning, etc.) and large-scale programs are expensive and labor intensive (Daane et al. 2008; Buczkowski et al. 2014a, b). Despite the consensus that currently available products for control of *L. humile* in agricultural settings are inadequate, little progress in commercial development of liquid bait delivery systems has been made. Significant factors impeding development of additional ant control technologies may include time to develop and test products, cost and registration constraints, and uncertainties over market size and adoption rates (Buczkowski et al. 2014b; Rust et al. 2015).

Currently, no *L. humile* treatment options have proven to be both consistently effective and economically viable for commercial agricultural operations. Previous research has focused largely on the evaluation of liquid bait compositions and application rates rather than the development of new modes of delivery suitable for large-scale, area-wide control programs. A handful of recent studies have evaluated synthetic polyacrylamide hydrogels (i.e., water storing crystals) as an alternative liquid bait delivery option for *L. humile* control (Buczkowski et al. 2014a, b; Rust et al. 2015; Boser et al. 2017; Merrill et al. 2018; Cooper et al. 2019). Porous hydrogels are conditioned in an insecticide-laced sucrose solution, and saturated beads act as miniature controlled-release bait dispensers (Tay et al. 2017). Hydrogels can be mass deployed with a mechanical spreader or aerial drop, forgoing the high labor output and expense associated with bait stations (Rust et al. 2015; Boser et al. 2017; Tay et al. 2017; Merrill et al. 2018; Schall et al. 2018; Cooper et al. 2019). Although polyacrylamide hydrogel applications have been reported to be highly effective in controlling *L. humile* in natural, urban, and agricultural settings, they are not readily biodegradable (Rust et al. 2015). Consequently, this hydrogel format may be unsuitable for commercial use.

To address the shortcomings of currently available treatment options for *L. humile* control, a non-toxic, biodegradable hydrogel was engineered from alginate, a naturally occurring polysaccharide found in brown seaweeds. In laboratory assays, the cross-linked calcium alginate gel matrix absorbed and delivered the targeted concentration of thiamethoxam within a liquid sucrose solution to *L. humile* (Tay et al. 2017). Alginate hydrogels were evaluated for control of *L. humile* in laboratory and urban field settings and demonstrated to be highly effective (Tay et al. 2017). To determine whether this alginate hydrogel bait delivery system could provide a comparable level of *L. humile* control in heavily infested citrus groves, a series of preliminary studies examining hydrogel application rates, application frequency, and dispersal methodology were conducted (Schall et al. 2018). These preliminary studies provided the basis for a three-month field study in which area-wide applications of alginate hydrogels were evaluated for control of *L. humile* in southern California commercial citrus orchards. Results of this large-scale field trial are presented here.

Materials and methods

Alginate hydrogel preparation

To produce calcium alginate hydrogels, 10 g L^{-1} sodium alginate (Na-Alg) solution was slowly poured into a 150-mm-diameter funnel attached to a 100-nozzle shower head (AKDY AZ-6021 8-inch bathroom chrome shower head, CA, USA) clamped to a retort stand. As the solution passed through the showerhead nozzles, droplets of 10 g L^{-1} Na-Alg solution formed. Droplets were collected in a 17-L plastic container filled with 5 g L^{-1} calcium chloride ($CaCl_2$) cross-linking solution that was continuously stirred to prevent the aggregation of beads. The resultant 60 kg of calcium alginate hydrogels was separated from the cross-linking solution, divided equally between three 68-L plastic storage bins each filled with 20 L of bait solution (50% sucrose solution with 0.0002% thiamethoxam [2 mg L^{-1} thiamethoxam]; 1:1 ratio of alginate hydrogel to liquid bait), and conditioned for a 24-h period to produce hydrated hydrogels containing a 25% sucrose solution with 0.0001% thiamethoxam (1 mg L^{-1}) (see Tay et al. 2017 for further detail). Thiamethoxam (Thiamethoxam PESTANAL®, Sigma-Aldrich) was the selected toxicant as it is dissolvable in sucrose solutions, non-repellent, consistently effective across a wide range of concentrations, and reported to be highly efficacious for ant control in a hydrogel-delivered

Table 1 Comparison of active ingredient concentrations, product application rates, and treatment costs for various Argentine ant control strategies used in California commercial citrus production

Application type	Trade name and company	Availability	Active ingredient and concentration	Dilution and concentration	Application rate; total product applied per ha	Amount of active ingredient per application per ha	Fold change in active ingredient versus chlorpyrifos in Lorsban-4E	Approximate cost of treatment[a]		
								Per kg or unit	Per month/ha	Per year/ha
Barrier spray	Lorsban-4E (Dow)	Commercial	44.9% chlorpyrifos	–	2.34 L	1050.66 mL	–	$14.53/L	$34.00	$408.00
Liquid bait	Gourmet liquid ant bait (Innovative Pest Control Products)	Commercial (organic)	1.0% disodium octaborate tetrahydrate	0.5% disodium octaborate tetrahydrate in solution	50 dispensers/ha[b] 0.5 L bait/dispenser 25 L bait/ha	0.125 mL	−8.41	Bait: $5.47/L Dispensers: $17.50/unit	Bait: $136.75 Dispensers: $875.00	Bait: $1641.00 Dispensers: $875.00 Total: $2516.00
Liquid bait	Optigard Flex[c] (Syngenta)	Experimental	21.6% thiamethoxam	0.0001% thiamethoxam in 25% sucrose solution	50 dispensers/ha[b] 0.5 L bait/dispenser 25 L bait/ha	0.025 mL	−42,026	Bait: $0.19/L Dispensers: $17.50/unit	Bait: $4.75 Dispensers: $875.00	Bait: $57.00 Dispensers: $875.00 Total: $932.00
Bait-loaded alginate hydrogels	Optigard Flex[c] (Syngenta)	Experimental	21.6% thiamethoxam	Hydrogels conditioned in a 0.0002% thiamethoxam, 50% sucrose solution to produce hydrogels containing 0.0001% thiamethoxam, 25% sucrose solution	0.25 kg hydrogel/tree[d] 280 trees/ha 70 kg hydrogel/ha	0.07 g	−15,009.43	Bait: $0.38 Hydrogel: $0.71/kg	Bait: $26.60 Hydrogel: $49.70	Bait: $319.20 Hydrogel: $596.40 Total: $915.60

Calculations are based on lowest-cost material sources available as of May 20, 2019. For pricing links and calculations, please see Online Resource 1

[a] Cost of treatment only does not include labor expenses. One application per month is assumed

[b] Application rate based on successful control of heavily Argentine ant-infested commercial citrus groves in southern California (94–99% reduction from baseline estimates for 18 months; McCalla unpublished data)

[c] Optigard Flex was substituted for analytical standard thiamethoxam in calculations for more cost-effective production of liquid bait

[d] Based on study rate

liquid sucrose bait (Buczkowski et al. 2014a, b; Rust et al. 2015; Tay et al. 2017; Boser et al. 2017; Merrill et al. 2018; Schall et al. 2018).

Bait-saturated hydrogels were sieved from the remaining liquid and condensed into one sealed storage bin to minimize air exposure and desiccation. For each batch of 60 kg of hydrogel, 360 mL of putative *L. humile* trail pheromone, (Z)-9-hexadecenal (microencapsulated formulation, 5.6 mg/mL; Suterra, LLC., Bend, OR) was added. The mixture was vigorously stirred for several minutes to ensure even distribution across hydrogels. The addition of (Z)-9-hexadecenal to toxicant-laced liquid bait has been shown to increase the rate of bait discovery by *L. humile* workers and control achieved (Welzel and Choe 2016). Hydrogels were immediately weighed out into 62.5 g aliquots and stored in 286-mL disposable plastic containers in a cold storage room (12 ± 2 °C) for up to 48 h before field deployment.

Field site and plot selection

Three commercial navel orange groves located in southern California (Redlands and Mentone, CA, USA) were selected for evaluation of the efficacy of alginate hydrogel applications for *L. humile* control. Twelve four-by-four (16) tree plots were selected per site, for a total of 36 plots. Plots were spaced at least 45 m apart to minimize movement of *L. humile* foragers between hydrogel-treated and control plots. Distance marking studies conducted in natural settings, vineyards, and citrus groves have reported that the vast majority of liquid bait movement by *L. humile* foragers occurs within 25–35 m of bait stations and seldom exceeds 45–50 m (Ripa et al. 1999; Vega and Rust 2003, Greenberg et al. 2006; Cooper et al. 2008; Hogg et al. 2018).

Following baseline assessments (see "monitoring" section), six of the twelve plots per site determined to have similar average *L. humile* activities were selected for experimental monitoring. Ant activity was surveyed on the four central trees of each plot, for a total of 18 plots (i.e., 72 trees) monitored in the study. Plots were randomly assigned to one of two treatments: hydrogel (i.e., 9 total plots; 36 total trees) or untreated control (i.e., 9 total plots; 36 total trees). This study did not include alginate hydrogels loaded with 25% sucrose water (no toxicant) as a treatment because this formulation was tested previously in Tay et al. (2017) and found to produce little to no mortality of laboratory colonies of *L. humile*. Due to potential contamination from an insecticide application at a nearby residence, ant monitoring in two plots (one treated, one control) at one site was discontinued. Monitoring in a third plot at a different site (treated) was discontinued due to an adjustment in irrigation schedule which offset watering and sampling of the plot from the other five. Maintenance of a consistent irrigation schedule for all plots within each site was critical to maintain consistency in hydrogel hydration following applications to soil (Schall et al. 2018). After these post-trial initiation adjustments, fifteen four-by-four tree plots and sixty trees (i.e., 32 trees and 8 plots in the control group and 28 trees and 7 plots in the treated group) were monitored for the full three-month study duration (August–October 2017).

Hydrogel applications

All sixteen trees in each plot randomly assigned to the treatment group received a series of three hydrogel applications each spaced 3 weeks apart at a rate of 250 g of hydrogel per tree. This rate was determined optimal in preliminary experiments (Schall et al. 2018). Hydrogel baits were hand-distributed on recently irrigated soil (< 48 h) surrounding experimental trees. To ensure even application, a 1.5-m-diameter circular hoop transect constructed from 3/4 in opaque vinyl tubing (Eastman Chemical Co., Kingsport, TN, USA) was divided into quarters with four pieces of flagging tape (Grainger Inc., Lake Forest, IL, USA). The hoop was placed around the base of each tree trunk, and flagging tape was extended from the margin of the hoop to the trunk to ensure consistency in treated area. Each flagging tape-delineated quadrant received one pre-weighed 62.5 g hydrogel aliquot, totaling 250 g of hydrogel across all four quadrants. After placement on soil, the hydrogel bead piles were hand-spread to ensure even dispersal. Summing all sites, 60 kg of hydrogel bait was applied per each of three treatment applications, for a total of 180 kg of hydrogel produced and applied throughout the experiment.

Monitoring

Monitoring was conducted over a three-month period from August 2017 to October 2017. *Linepithema humile* activity was assessed in a subset of the four innermost trees of each plot across three baseline samplings (i.e., 1 week, 2 days, and 1 day prior to the first hydrogel application) and fifteen post-baseline samplings (i.e., 24 h, 48 h, 1 week, 2 weeks, and 3 weeks following each of the three hydrogel applications). Two methods were utilized to monitor ant activity: (1) 1-min visual estimations of the number of workers ascending and descending each tree trunk, and (2) 24-h deployments of two monitoring vials each filled with 40 mL of 25% sucrose solution at the base of monitored trees. Monitoring vials were constructed from Falcon 50-mL conical centrifuge tubes (Corning Inc., Corning, NY, USA) fitted with a 40 × 40 mm square of weed block fabric (Easy Gardener Products Inc., Waco, TX, USA) secured between the vial opening and lid. A 2.5-mm-diameter hole was made in each lid to allow ant access to bait which was imbibed through the weed block fabric. To estimate *L. humile* activity with baited monitors, the pre- and post-deployment weights of each vial were compared. After accounting for evaporative loss (i.e., liquid loss in deployed control vials with no ant access), the amount of liquid consumed was divided by 0.003 g (i.e., the average amount of liquid removed by each *L. humile* forager per visit) to determine the total number of ant visits (detailed methods in Reierson et al. 1998).

In addition to monitoring *L. humile* activity, soil temperature and moisture data were collected with a digital soil thermometer (Model S40P-V; Dr. Meter Co. Ltd., Kaohsiung City, Taiwan) and moisture meter (Model HSM50; Omega Engineering Inc., Norwalk, CT, USA). For each monitored tree on all sampling dates, meters were deployed in the soil near the trunk for 2 min at a depth of approximately 2.5 cm.

Statistical methods

A linear mixed model ("*lme*" function in the lme4 package, R) was used to examine the effect of hydrogel treatment (fixed, categorical), monitoring date (fixed, continuous), their interaction, and the covariates soil temperature (fixed, continuous) and soil moisture (fixed, continuous) on visual and baited vial estimations of *L. humile* activity. A nested error structure (i.e., trees within plots within sites) was used to account for the pseudoreplication inherent in a hierarchically organized experimental design (Spurgeon 2019). Model selection was conducted through backwards elimination and comparison of AIC values, with lower values indicating superior model fit. Ant activity data were power transformed to satisfy normality and homoscedascity assumptions prior to analysis. The overall difference in ant activity between hydrogel-treated and control trees across combined sampling dates was examined using the Tukey-adjusted estimated marginal means function ("*emmeans*" in the emmeans package, R). All analyses were conducted in the statistical software R, and comparisons were considered significant if $P < 0.05$ [version 3.5.2, R Development Core Team (2018)].

Mean baited vial and visual estimates of *L. humile* activity collected 48 h after each hydrogel treatment and on the final sampling date were compared across treatment type (i.e., control or hydrogel treated) and to the baseline estimate (averaged across all baseline sampling days) using a linear mixed model. Ant activity data were power transformed to meet normality and homoscedascity assumptions prior to analysis. Hydrogel treatment, treatment period (i.e., baseline, 48 h after the first, second, and third treatments, and the final sampling point), and their interaction were included as fixed categorical factors, soil temperature was included as a fixed continuous covariate, and the nested error structure was defined as trees within plots within sites. Ant activity was compared between treatment groups and pre- and post-treatment with Tukey-adjusted estimated marginal means.

To evaluate the burden of cumulative *L. humile* infestation, power-transformed *L. humile* activity data (visual) were converted to insect days (ID) and then summed across the entire sampling period (cumulative insect days; CID) with the following formulas:

$$\mathrm{ID} = 0.5\left(D_a + D_b\right)\left(T_a - T_b\right)$$

$$\mathrm{CID} = \sum \mathrm{ID}$$

where D_a and D_b are corresponding *L. humile* densities at adjacent time points T_a and T_b (which ranged from 1 to 7 days in this study) (Ruppel 1983). Insect days were summed across all sampling dates to calculate cumulative insect days, a measure of the intensity of cumulative *L. humile* infestation on each tree over the study duration. Mean cumulative *L. humile* activities for hydrogel-treated and control trees were compared between treatment groups with a linear mixed model. Treatment, monitoring date, and their interaction were fixed factors.

Results

For both visual and baited vial estimates of *L. humile* activity, soil moisture was not found to be a significant model covariate ($T_{1,816} = 0.051$, $P = 0.61$ and $T_{1,767} = 1.50$, $P = 0.13$ respectively) and was dropped from the final model. It is possible that inconsistent irrigation timing among plots or the depth of soil sampling may have confounded this effect. Hydrogel treatment ($T_{1,817} = -3.73$, $P < 0.01$), the interaction between treatment and monitoring date ($T_{1,817} = -7.70$, $P < 0.0001$), and the covariate soil temperature ($T_{1,817} = -2.98$, $P < 0.05$) significantly affected visual estimates of mean *L. humile* activity (i.e., positive correlation between ant activity and soil temperature). Monitoring date alone ($T_{1,817} = -1.0$, $P = 0.28$) was not a significant model factor. Baited vial estimates of mean *L. humile* activity followed a similar pattern, with a significant effect of treatment ($T_{1,768} = -2.98$, $P < 0.05$), monitoring date ($T_{1,768} = 2.93$, $P < 0.01$), their interaction ($T_{1,768} = -2.07$, $P < 0.05$), and soil temperature ($T_{1,768} = 6.98$, $P < 0.0001$). Mean *L. humile* activity was significantly lower in treated trees than control trees for both visual ($T_{1,817} = 7.57$, $P < 0.0001$) and baited vial ($T_{1,768} = 4.72$, $P < 0.001$) estimates (Fig. 1; Table 2).

Baseline *L. humile* activities were not significantly different between treated and control trees for visual ($T_{4,\,351} = 0.60$, $P = 1.00$) or vial estimates ($T_{269} = -0.78$, $P = 1.00$) (Table 2; Fig. 1). Visual observations of ant activities were significantly lower in treated trees than control trees 48 h following all hydrogel applications ($T_{4,\,351} = 5.54$, $P < 0.01$; $T_{4,\,351} = 4.94$, $P < 0.05$; $T_{4,\,351} = 13.79$, $P < 0.0001$) and on the final sampling date ($T_{4,\ 351} = 5.67$, $P < 0.01$). Estimated ant activities from monitoring vial data for treated trees were significantly lower than control trees following the first two hydrogel treatments ($T_{269} = 4.53$, $P < 0.05$; $T_{269} = 8.92$, $P < 0.0001$) but not significantly different after the final treatment ($T_{269} = 3.77$, $P = 0.06$) or on the last sampling date ($T_{269} = 2.32$, $P = 0.45$).

A comparison of visual and vial estimations of *L. humile* activity prior to and following successive hydrogel applications is provided in Table 2 and Fig. 1A. Visual counts indicated a significant 40, 79, and 91% reduction from baseline activity 48 h after the first ($T_{4,\ 351} = -4.66$, $P < 0.001$), second ($T_{4,\ 351} = -7.75$, $P < 0.0001$), and third hydrogel applications ($T_{4,\ 351} = -16.53$, $P < 0.0001$), respectively. Concurrently, visual *L. humile* activity increased in control trees with a 34 ($T_{4,351} = 2.85$, $P = 0.12$), 34 ($T_{4,\ 351} = -1.84$, $P = 0.71$), and 51% increase ($T_{4,\ 351} = 2.40$, $P = 0.33$) from baseline estimates following each application. These increases, however, were not significant. Three weeks after the final application, visual *L. humile* activity in treated trees was 67% lower than baseline estimates ($T_{4,\ 351} = -9.96$, $P < 0.0001$) and visual activity in control trees was not significantly different from baseline estimates ($T_{4,\ 351} = -2.90$, $P = 0.11$), indicating considerable residual activity of hydrogel treatments.

Although baited vial estimates of *L. humile* activity in treated trees followed a trend similar to visual estimates, the greatest percent reduction from baseline values was seen at 24 h (i.e., a 78, 77, and 81% reduction following the first, second, and third treatments, respectively) rather than 48 h after hydrogel application (Fig. 1B). An insignificant 48% increase ($T_{269} = -0.65$, $P = 1.00$), significant 41% decrease ($T_{269} = -4.18$, $P < 0.01$), and insignificant 30% decrease ($T_{269} = -0.59$, $P = 1.0$) in ant activity were observed in treated trees 48 h after the first, second, and third hydrogel applications, respectively. Concurrently, ant activity in control trees rose, with an observed 206 ($T_{269} = 7.33$, $P < 0.0001$), 227 ($T_{269} = 9.52$, $P < 0.0001$), and 120% increase ($T_{269} = 6.00$, $P < 0.0001$) from baseline estimates (Table 2). Vial estimates of *L. humile* activity in treated trees on the final sampling date did not corroborate the residual bait efficacy indicated by visual estimates. Three weeks after the final treatment, vial estimates were 53 and 235% higher than baseline values in treated ($T_{269} = 5.22$, $P < 0.0001$) and control trees ($T_{269} = 9.30$, $P < 0.0001$), respectively.

Mean cumulative infestation burden of *L. humile* (as determined by visual estimates) in hydrogel-treated trees (9020.64 ± 650.45 CID) was less than half that of control trees (22,714.83 ± 1967.40 CID) (Fig. 2). Hydrogel treatment ($T_{1,817} = -5.22$, $P < 0.01$), the interaction between treatment and monitoring date ($T_{1,817} = -6.98$, $P < 0.0001$), and the covariate soil temperature ($T_{1,817} = -1.19$, $P < 0.05$) were all significant model factors.

Discussion

Current treatment options for managing Argentine ant populations in commercial citrus are inadequate. Chlorpyrifos barrier sprays repel or provide temporary knockdown of *L. humile* foragers but are ineffective in controlling subterranean ant colonies, require frequent reapplication, and are hazardous to human, natural enemy, and environmental health (Knight and Rust 1990a; Rust et al. 1996; Thomson and Hoffmann 2006; Daane et al. 2008; California Department of Pesticide Regulation 2019a, b). Liquid baiting programs preserve biological control agents and provide colony-level control of *L. humile* (Cooper et al. 2008; Schall and Hoddle 2017). However, plastic bait stations must be placed in high densities to be effective, and the cost to purchase and time to deploy and service dispensers make baiting a potentially undesirable management option for growers maintaining large operations (Nelson and Daane 2014; Schall et al. 2018). To generate industry interest and end-user adoption of research-developed bait and bait delivery products, treatments must be targeted, effective, economically viable, and easily deployed at a commercial scale.

Hydrogels are a recently explored format for mass delivery of toxicant-laced aqueous bait to pestiferous sugar-feeding ants such as *L. humile*. However, previously studied matrix compositions (i.e., polyacrylamide) degrade into toxic components (Xiong et al. 2018) and, consequently, are unlikely to be registered for commercial use (Tay et al. 2017). To produce a product suitable for *L. humile* control in commercial agriculture, a biodegradable alginate hydrogel was engineered and shown to be highly effective in laboratory assays, field trials in urban areas (Tay et al. 2017), and preliminary field trials in citrus orchards (Schall et al. 2018).

In the present study conducted in commercial citrus groves, both visual and baited vial estimates indicated area-wide applications of alginate hydrogels produced excellent control of *L. humile* despite the presence of heavy infestations in neighboring untreated plots. Excluding the baseline and averaging across all sites and sampling dates, mean *L. humile* activities (visual and vial) and cumulative infestation in hydrogel-treated trees were significantly lower than in control trees. Forty-eight hours after each hydrogel application, mean *L. humile* activities (visual and vial) were lower in hydrogel-treated trees than in control trees. However, the overall trend for pre- and post-treatment ant activities diverged for visual and vial estimates.

According to visual estimates, successive hydrogel treatments increased overall *L. humile* control achieved and reduced the rate of activity rebound in treated trees. A significant 40% reduction in ant activity from baseline estimates was observed following the initial hydrogel application. However, ant activity rapidly increased after the 48-hour

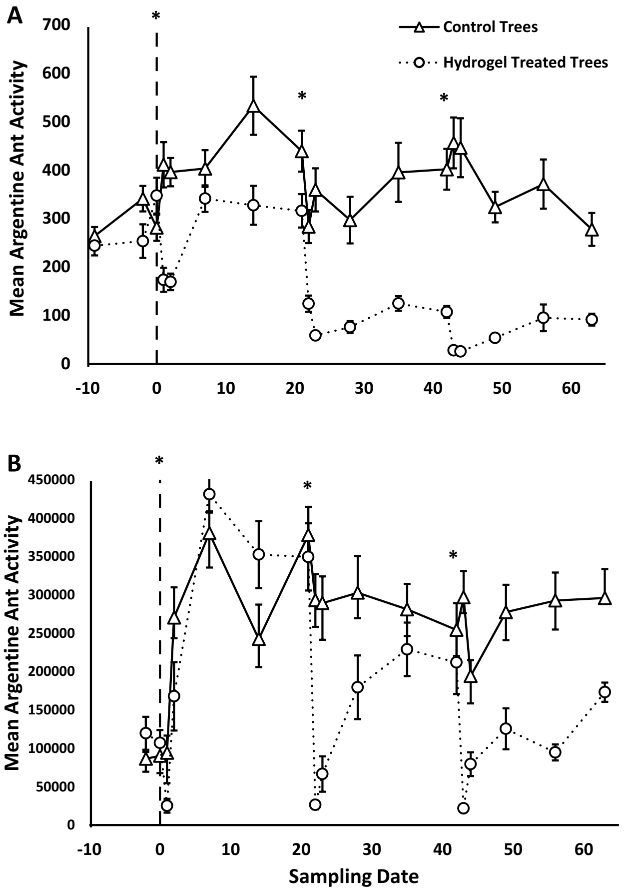

Fig. 1 Mean visual (number of ants traversing tree trunks per 1-min observation; **A** and 24-h baited vial (number of ant visits to baited monitors over a 24-h period; **B** estimates (±SEM) of foraging Argentine ants in hydrogel-treated and control trees averaged across all sites. The dashed line separates pre- and post-baseline sampling dates. Asterisks denote hydrogel application dates

point, returning to pre-treatment levels within a week. The second hydrogel application further reduced ant activity (a significant 79% lower than baseline estimates) and resulted in a slower activity resurgence. Peak control was achieved 48 h after the third and final hydrogel application, with a recorded 17-fold difference in *L. humile* activity between treated and control trees and a 91% reduction in activity from baseline estimates. In treated trees, ant activity was significantly lower (nearly 70%) than baseline levels 3 weeks after the final treatment, a residual lifespan similar to that of chlorpyrifos barrier sprays (Knight and Rust 1990a; Rust et al. 1996).

The improved residual efficacy of the second and third hydrogel applications relative to the first is unsurprising, given the extraordinarily high densities of *L. humile* reported to infest southern California citrus groves (Markin 1970; Schall and Hoddle 2017). As local nests are eradicated in bait treatment areas, *L. humile* from neighboring colony districts reinvade unoccupied zones for resources, resulting in rapid population rebound (Vega and Rust 2003; Nelson and Daane 2014; Schall et al. 2018). Waves of reinforcement foragers and their respective colonies are eliminated with subsequent treatments, producing long-lasting area-wide control. Thus, initial hydrogel applications may act as a "sink," the extent to which is dependent on the severity and extent of *L. humile* infestation and spatial resource saturation. Resurgence in ant activity is likely to be more rapid in heavily infested areas (i.e., large, homogenous swathes of untreated citrus production area). A shorter initial reapplication interval could facilitate elimination of colonies in reinvaded treatment areas. In the current study, the treatment of small plots surrounded by heavily infested untreated areas increased reinvasion pressure. Replication and the inability to mass-produce hydrogels in quantities sufficient for area-wide application necessitated this experimental design. However, in a real-world scenario, hydrogels would be dispersed across entire groves, eliminating the majority of *L. humile* reservoir populations. Consequently, ant activity rebound amplitude and reapplication interval may be further reduced with commercial-scale hydrogel applications (Schall et al. 2018).

In comparison with visual counts, vial sampling methods recorded smaller improvements in *L. humile* control with successive hydrogel treatments and a more rapid resurgence in activity following all applications. Vial estimates of activity between treated and control trees were significantly different 48 h after the first two hydrogel applications (Table 2). Ant activity was recorded to be the lowest 24 h after each application (i.e., 78, 77, and 81%, respectively) and began to rebound 48 h after each application (Fig. 1). The consistent level of reduction observed at the 24-h point is likely the result of forager disruption by hydrogel applications, as the bait evaluated has a delayed toxic effect requiring 2 to 3 days to produce high worker mortality (Tay et al. 2017). The divergence in baited vial and visual ant activity trends 24 and 48 h after treatments may be related to fundamental differences between these monitoring techniques.

In studies where ant activity is monitored, baited vial estimates are commonly used to corroborate visual estimates. However, these sampling measures provide estimates of different niches (i.e., visual estimates measure canopy activity and vial estimates measure ground activity) and are subject to method-inherent biases. Manually counting workers traversing irrigation pipes or tree trunks is subject to human error and provides only a "snapshot" of activity based on the duration of sampling time and the time of day at which observations are made. Consequently, variation in activity may be missed (i.e., at night when more than half of *L. humile* activity occurs) (Agosti et al. 2000; Kistner et al. 2017). While sampling with baited vial monitors can account for changes in ant activity over time

Table 2 Comparison of mean Argentine ant activities (±SEM) as estimated visually (number of ants traversing tree trunks per 1-min observation) and with baited vials (number of ant visits to baited monitors over a 24-h period) on hydrogel-treated and control citrus trees across all sites following applications

	Average of all baseline sampling dates	48 h after first application (day 2)	48 h after second application (day 23)	48 h after third application (day 44)	Final sampling date; 3 weeks after third application (day 63)
Mean Argentine ant activity: visual estimates					
Control activity (percent change from baseline)	295.33 ± 14.28^{aA}	395.69 ± 29.20^{aA} (+34.0%)	395.06 ± 44.48^{aA} (+33.8%)	445.97 ± 60.79^{aA} (+51.0%)	277.63 ± 33.91^{aA} (−6.0%)
Hydrogel treatment activity (percent change from baseline)	281.61 ± 18.66^{aA}	169.39 ± 17.12^{bB} (−39.9%)	59.14 ± 7.88^{bB} (−79.0%)	25.89 ± 3.59^{bB} (−90.8%)	91.57 ± 12.22^{bB} (−67.5%)
Mean Argentine ant activity: baited vial estimates					
Control activity (percent change from baseline)	88,376.36 ± 9593.92^{aA}	270,311.51 ± 39,450.78^{aA} (+205.9%)	289,210.86 ± 34,805.22^{aA} (+227.3%)	194,178.75 ± 20,759.58^{aA} (+119.7%)	295,814.47 ± 37,784.38^{aA} (+234.7%)
Hydrogel treatment activity (percent change from baseline)	113,429.92 ± 13,593.44^{aA}	167,726.32 ± 44,585.26^{bA} (+47.9%)	66,488.29 ± 23,072.65^{bB} (−41.4%)	79,362.91 ± 15,449.85^{aA} (−30.0%)	173,108.49 ± 12,556.10^{aA} (+52.6%)

Within each column (control vs. treatment), means (±standard error) followed by the same lowercase letter are not significantly different at $\alpha = 0.05$ (estimated marginal means). Within each row (pre- vs. post-treatment), means followed by the same uppercase letter are not significantly different at $\alpha = 0.05$ (estimated marginal means). Values in parentheses represent percent change from baseline estimates

(Reierson et al. 1998), provision of an additional food source artificially inflates estimates as *L. humile* foragers proportionally recruit to resources (Agosti et al. 2000; Silverman and Brightwell 2008). This is particularly problematic when sampling alongside other competing attractants such as the hydrogels used in the present study. The addition of a large, highly palatable food source may have attracted new foragers and redirected nearby foragers collecting sugar water away from baited vials, resulting in an underestimation of activity at the 24-h mark. However, following hydrogel depletion (i.e., ~86% of the weight of field-deployed hydrogels is lost within a 24-h period [Schall et al. 2018]), all residual foragers may have aggregated to baited vials, inflating estimates of activity at the 48-h mark. Consequently, ground-deployed baited vials may be an unreliable sampling method for monitoring *L. humile* activity when paired with attractant-based treatment programs. The development of ant monitoring technologies (e.g., infrared sensors) capable of capturing hourly fluctuations in activity over a 24-h period without artificially inflating counts could effectively replace baited vial sampling.

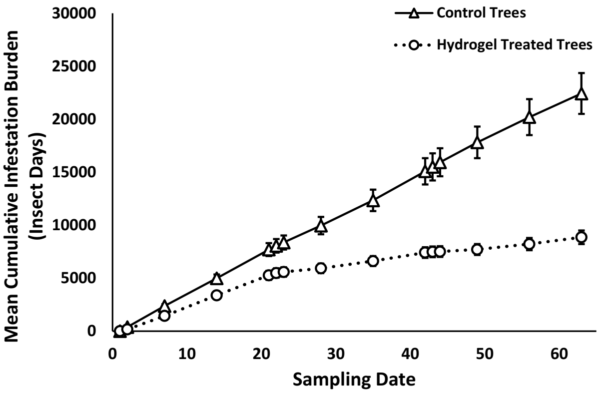

Fig. 2 Mean cumulative *L. humile* infestation burden in hydrogel-treated and control trees (±SEM) across all sampling dates

The results presented here suggest alginate hydrogels loaded with thiamethoxam-laced aqueous bait are a highly effective and sustainable alternative treatment option to chlorpyrifos spray programs in California citrus for *L. humile* control. California has recently banned chlorpyrifos due to its designation as a toxic air contaminant and all usage will cease by 2021 (Environmental Protection Agency 2016; California Department of Pesticide Regulation 2019a, b). The mammalian toxicity of thiamethoxam is 4.5–19 (oral) and > 6.7 (inhalation) times lower than chlorpyrifos (National Center for Biotechnology Information PubChem Database). Furthermore, the concentration of thiamethoxam in a hydrogel application is more than 15,000 times lower than that of chlorpyrifos in a Lorsban-4E application (Table 1). In these amounts, thiamethoxam presents a negligible human health risk. Even if the thiamethoxam in a single hydrogel application at the study rate (i.e., 70 kg/ha of hydrogel and 0.07 g/ha of thiamethoxam) leeched into the soil, was absorbed by tree roots, and was expressed in the fruit without any loss, the total 0.00084 ppm of thiamethoxam is just 0.21% of the Environmental Protection Agency's (EPA) 0.4 ppm fruit tolerance level in conventional citrus production. At such trace quantities, this treatment would even meet organic citrus production regulations (i.e., one application registers at just 4.2% of the EPA's 0.02 ppm fruit tolerance level) where a small quantity of pesticide is expected from contaminated water or soil sources and pesticide drift.

Although neonicotinoids including thiamethoxam have been implicated in pollinator and natural enemy declines in agroecosystems (Prabhaker et al. 2011; Blacquiere et al. 2012; Stanley et al. 2015), the small quantity of thiamethoxam used in hydrogel applications is unlikely to affect beneficial arthropods present in citrus. Furthermore, the inclusion of putative *L. humile* trail pheromone (Z-9-hexadecenal) in the bait formulation, the short one- to three-day period hydrogels remain hydrated and palatable, the guarding of hydrogels by *L. humile* workers, and the transport of bait to inaccessible subterranean ant colonies all minimize potential off-target effects (Schall et al. 2018). Consequently, hydrogel treatments are unlikely to impact biological control and could be a highly effective component of integrated pest management programs for sugar-feeding ants and the sap-feeding pests they tend.

The economic feasibility of hydrogel transport of aqueous bait is an improvement over traditional liquid baiting programs for *L. humile*, which necessitate initial investment in bait dispensers and continuous labor costs for servicing. The material costs for a year of hydrogel applications is less than half that of commercial liquid baiting programs utilizing dispensers (Table 1). Alginate hydrogels are produced from natural, inexpensive, commercially available materials and require minimal labor input and maintenance, as beads can be rapidly hand-distributed or broadcasted through a large mechanical fertilizer spreader or aerial drops (Tay et al. 2017; Merrill et al. 2018; Schall et al. 2018; Cooper et al. 2019). To minimize the risk of spoilage and reduce the weight and cost of transport, alginate hydrogels could potentially be stored and dispersed in a dry format and "activated" in the field with irrigation water. The viability of this application method should be further investigated. Alginate hydrogels have ease-of-use comparable to chlorpyrifos barrier sprays and provide excellent colony-level control using toxicant concentrations several orders of magnitude lower. These features make the alginate hydrogel an excellent candidate for commercial mass production and agricultural application where control of sugar-feeding pest ants is needed.

Conclusions

The alginate hydrogel baiting system joins the area-wide, colony-level ant control and integrated pest management synergy of standard bait-and-dispenser strategies with the low-labor and cost of broad-spectrum insecticide sprays to provide a comprehensive, sustainable baiting product suitable for control of *L. humile* in commercial citrus production. Future studies could evaluate the efficacy of alginate hydrogels for use against other pestiferous sugar-feeding ant species or in other crops such as grapes which suffer economically significant damage from ants and ant-tended hemipteran pests.

Author contributions

All authors helped conceive and provided input on experimental design. JT, DC, and AM developed the alginate hydrogel bait and oversaw production of product for field deployments. KM and MH conducted the field trial. KM ran analyses on collected data and wrote the manuscript. All authors reviewed, provided feedback, and approved of the manuscript.

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Compliance with ethical standards

Conflict of interest All authors declare they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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