

Household and Structural Insects

Development and Demonstration of Low-Impact IPM Strategy to Control Argentine Ants (Hymenoptera: Formicidae) in Urban Residential Settings

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Abstract

Argentine ants are one of the most common nuisance pest ants treated by pest management professionals (PMPs) in southern and western urban residential areas of the United States. Two new technologies (spraying with a pheromone adjuvant and using a biodegradable hydrogel bait delivery method) were used to develop a unique low-impact integrated pest management (IPM) protocol for Argentine ants in urban residential settings. The IPM protocol included a one-time perimeter spray treatment with 0.03% fipronil (mixed with a pheromone adjuvant) at the beginning of the ant season to achieve a quick knockdown. The initial spray application was followed by a biodegradable hydrogel baiting with 1% boric acid as a maintenance treatment. This low-impact IPM protocol was compared with two other conventional methods: (1) one initial fipronil application and one pyrethroid spray application for maintenance, or (2) one initial fipronil application and one essential oil insecticide spray application for maintenance. Based on Argentine ant foraging activity, the protocols were compared for their control efficacy. Insecticide use information and treatment time were also recorded and compared among different treatment protocols. Our results provided empirical data to support the effectiveness and economic feasibility of the low-impact IPM protocol for managing Argentine ants in urban residential settings.

Key words: Fipronil, boric acid, perimeter treatment spray, adjuvant pheromone, hydrogel ant bait

In urban residential areas of the southern and western United States, the Argentine ant, *Linepithema humile* (Mayr), is one of the most common nuisance ant species treated by pest management professionals (PMPs) (Silverman and Brightwell 2008). Due to their ease of application and cost-effectiveness, contact and residual insecticide sprays are commonly applied for outdoor Argentine ant control. However, many of the active ingredients used in these insecticides are frequently detected in urban waterways (Greenberg et al. 2014, references cited therein; Greenberg and Rust 2019). Insecticides applied around residential areas to control ants and other peridomestic pests are recognized as one of the major sources of these contaminants. In an effort to reduce insecticide runoff, several regulatory changes and label updates have been made. For example, the California Department of Pesticide Regulation (CA DPR) issued Urban Surface Water Protection Regulations for various products

containing pyrethroids (effective July 2012, CA DPR 2012). Also, the U.S. Environmental Protection Agency (U.S. EPA) recently approved new label amendments (approved April 2017) for fipronil products (US EPA 2017). According to the California Specific Use Restrictions in the new label, fipronil application to driveways and garage door areas are prohibited. All fipronil applications during the rainy season (from November 1 to February 28) are prohibited. The finished dilution rate for fipronil was reduced from 0.06% to 0.03%. While short- and long-term outcomes of these new regulations in reducing the pesticide runoff have yet to be determined, these changes will certainly impact current general pest control practices around structures, especially ant control methods. Thus, it is critical to develop improved control strategies in which smaller amounts of insecticides and low-impact materials still achieve satisfactory levels of ant control.

One possible approach to improve insecticide efficacy and potentially reduce overall insecticide use is incorporating a synthetic Argentine ant pheromone in the insecticide sprays. The addition of the Argentine ant's synthetic trail-following pheromone, (Z)-9-hexadecenal, in the insecticide spray improved the control efficacy by attracting the ants to the spray deposits (Choe and Campbell 2014, Choe et al. 2014). This 'lure and kill' approach has the following advantages over conventional standalone applications of insecticide sprays: (1) The insecticide/pheromone treated surface (soil, cement, wood) attracts foraging ants from nearby trails and even from the nest, thus maximizing the number of exposed ants, potentially reducing the need for additional treatments and risk of runoff (Greenberg and Rust 2019). (2) It maximizes the exposure of individual ants to insecticide spray deposits before any significant degradation of the active ingredient occurs due to exposure to sunlight or precipitation. In recent field evaluations by a multinational pest management company, adding the pheromone in the first fipronil application for Argentine ant control dramatically reduced (by about 60%) the call back rate (i.e., failure of the control, and the company providing a free follow-up application of the pesticide) (Pat Copps, personal communication).

Another proven method in ant control to reduce insecticide use and potential runoff is baiting. Liquid baits are an effective alternative to insecticidal sprays to control several sugar-feeding ant species, including Argentine ants (Klotz et al. 2003, 2004; Greenberg et al. 2013). However, liquid baits require bait stations which are typically expensive and labor-intensive to maintain. In addition, PMPs are reluctant to leave bait stations around structures. To overcome these limitations, we recently developed a biodegradable alginate hydrogel matrix to deliver a liquid bait targeting pestiferous sugar-feeding ants such as Argentine ants (Tay et al. 2017). The beads of alginate hydrogel (derived from seaweed) were engineered by optimizing a cross-linking process and mass-produced in the laboratory. The hydrogel beads were then conditioned (24 h) in 25% sucrose bait containing a small amount of toxicant (thiamethoxam). The highly absorbent hydrogel matrices act as a controlled-release vehicle as they keep the liquid bait palatable for the ants. Based on recent field trials, the biodegradable alginate hydrogel bait with 0.0001% thiamethoxam provided excellent control of Argentine ant field populations (Tay et al. 2017, 2020; McCalla et al. 2020).

In the current study, we developed and field tested a low-impact IPM protocol for Argentine ant management in urban settings using two aforementioned approaches (i.e., pheromone adjuvant for spray applications and biodegradable hydrogel bait). In the first part of the project, we investigated if biodegradable hydrogel baits with boric acid (1%) as an active ingredient would provide substantial reductions of Argentine ant activity during their typical peak season in urban California (August–September). Since the hydrogel beads containing liquid bait were to be applied around houses, the relatively low toxicity of boric acid to nontargets was an important consideration. Boric acid is practically nontoxic to birds, fish and aquatic invertebrates, and relatively nontoxic to beneficial insects (US EPA 1993). According to the EPA toxicity rating standard (acute oral toxicity), boric acid is rated 'toxicity category III', indicating it is only slightly toxic for vertebrates (US EPA 2006). However, boric acid is an effective toxicant for Argentine ants when it is incorporated in a liquid bait (Klotz et al. 2000, Daane et al. 2008).

In the second part of the project, the low-impact IPM protocol was compared with two other methods that mimic the conventional ant treatment protocols of PMPs. A one-time perimeter treatment with fipronil spray was incorporated in all the protocols. The initial spray application was followed by one follow-up maintenance

treatment at week 4. Ant foraging activity levels were monitored throughout the season (July–October) and compared among different treatment protocols. Additionally, the amount of insecticide applied and the time required to apply the treatments were compared between different treatment protocols. The goal of this study was to determine effectiveness and economic feasibility of the low-impact IPM protocol in controlling Argentine ants around houses compared with the two conventional protocols.

Materials and Methods

Experimental Settings

Residential houses in Riverside, CA, USA, were used for the experiments. In 2017, four houses were used to determine the effectiveness of a one-time treatment of boric acid hydrogel baits, each house representing one replicate. In 2019, three protocols (two conventional protocols and one low-impact IPM protocol) were tested. Five houses were assigned to each of three protocols, each house representing one replicate. For both years, the foraging activity level of ants (number of ant visits) was estimated based on the total amount of sucrose solution consumed over a 24-h period (Welzel and Choe 2016). The average value from 10 monitoring sites placed around the foundation was used for statistical analyses. To understand the overall Argentine ant activity in the absence of treatment efforts, an untreated control house was also monitored during the entire project period for each year.

2017 Study (boric acid hydrogel bait)

One-time treatment of 1% boric acid hydrogel baits was made at residential houses to determine its efficacy in Argentine ant control. The biodegradable hydrogel bait was produced by the method described by Tay et al. (2017) with minor modifications. The 1% sodium alginate solution (Na-Alg) was slowly dispensed dropwise through a modified 20.3-cm (diameter) shower head with 90 nozzles (1.6 mm diameter). The droplets were immediately collected in a plastic container with 0.5% CaCl_2 crosslinker solution. After 2 min, the resulting hydrogel beads were filtered out from the crosslinking solution and rinsed with clean tap water. The rinsed hydrogel beads were 'conditioned' by submerging them in a solution containing sucrose and boric acid overnight (24 h). The concentrations of the sucrose and boric acid in the final, conditioned hydrogel bait were 25 and 1%, respectively. A pheromone adjuvant (microencapsulated (Z)-9-hexadecenal 0.56%; Suterra, LLC., Bend, OR; 1 ml per liter of bait) was mixed with the final hydrogel bait immediately before application. The addition of the pheromone adjuvant in the baits was expected to facilitate the discovery and consumption of the baits by the Argentine ant foragers (Welzel and Choe 2016). Each house was treated (late August) with approximately 3 liters of hydrogel baits on the ground by hand-tossing, mostly on active ant trails, within 5 m of the building.

2019 Study (conventional protocols)

We tested two different conventional protocols that mimicked ant treatment protocols used by PMPs. Both conventional protocols consisted of a one-time 0.03% fipronil spray treatment (Termidor SC, BASF, Research Triangle Park, NC) in summer (late July), followed by a maintenance treatment with another spray product at week 4 post-treatment (Table 1). For the maintenance treatment, conventional protocol #1 used a 0.06% bifenthrin spray (Talstar P, FMC Corp., Philadelphia, PA) and conventional protocol #2 used a botanical insecticide spray containing a mixture of rosemary oil, geraniol, peppermint oil, and wintergreen oil (Essentria IC3, Central

Garden & Pet Company, Schaumburg, IL). The maintenance treatment focused on active ant trails on soil, lawn, and other horizontal surfaces within 5 m of the building. All spray products were prepared and applied with a backpack sprayer (Birchmeier Iris 15, Stetten, Switzerland) following the label recommendations. The initial fipronil treatment was made in late July, and the maintenance treatment was made in late August (week 4).

2019 Study (low-impact IPM protocol)

The low-impact IPM protocol consisted of a one-time 0.03% fipronil spray (mixed with the pheromone adjuvant, microencapsulated (Z)-9-hexadecenal; 25 ml per 3.8 liters of spray) in summer (late July) followed by the biodegradable hydrogel bait conditioned with 1% boric acid at week 4 post-treatment as a maintenance treatment (Table 1).

The biodegradable hydrogel bait was produced using the same method as the protocol used in the 2017 study. To improve the stability (i.e., shelf life) of the final hydrogel bait, 0.25% sorbic acid potassium salt was incorporated in the final hydrogel bait. The pheromone adjuvant (microencapsulated (Z)-9-hexadecenal; 1 ml per liter of bait) was also mixed with the hydrogel bait immediately before application.

The hydrogel bait was scattered on the ground using a manual or motorized spreader, mostly on active ant trails, soil, or vegetated surfaces within 5 m of the building. As in the conventional protocols, the bait was not applied to horizontal surfaces such as concrete, asphalt, and bricks. The application rate was 4–8 liters/100 m² (1–2 gal/1,000 ft²). Depending upon the individual ant pressure and configuration of yards, about 4–7 liters of hydrogel bait was applied at each house (approximately 40–70 g boric acid per house).

Data Collection and Statistical Analyses

In the 2017 study, a one-time boric acid hydrogel bait application was made at each house. The treated houses were monitored on day 1 pretreatment, and weeks 1, 2, 3, and 4 after the treatment.

For the 2019 study with the conventional protocols and the low-impact IPM protocol, the sites were monitored once before the treatment, and weeks 1, 2, and 4 after the treatment. After the maintenance treatment in week 4, the sites were further monitored at weeks 5, 6, and 8. For each treatment, the amount of spray and bait applied (in liters) and the time required to make the applications were recorded.

For both 2017 and 2019 data, a Friedman test, a nonparametric alternative to a one-way repeated-measures analysis of variance (Kim 2014), was used to assess differences in ant visits (average value from 10 monitoring sites) between different monitoring time points within a treatment protocol. If the Friedman test indicated a significant difference among different monitoring time points, Conover all-pairwise comparisons test was used to compare ant visit numbers between all pairs of monitoring time points. For the 2019

study, a Kruskal–Wallis test was used to compare three groups of houses in their pretreatment ant activity levels (Analytical Software 2008). Data from one untreated house (not replicated) were used to show natural seasonal ant activity for both years, but not used for the statistical analyses.

Results

Control Efficacy

For the 2017 study, the pretreatment ant visit number was 55,125 ± 7,816 (mean ± SEM). The ant visit numbers after the boric acid hydrogel bait treatment were 26,519 ± 11,247, 36,383 ± 8,202, 39,460 ± 16,260, and 7,308 ± 3,026 per monitoring vial (mean ± SEM) for week 1, 2, 3, and 4 post-treatments, respectively. The ant visit numbers at the four houses showed a significant decline over time, at day 1 pretreatment and week 4 post-treatment (Friedman test: $F = 13.00$, $P = 0.01$; Fig. 1). During the entire 4-wk study period, the untreated control house did not show any drop in ant activity level. For example, the untreated control house recorded 10,440 ant visits in pretreatment (late August), 43,342 in week 1, 61,515 in week 2, 30,783 in week 3, and 17,260 in week 4, showing a 65–489% increase of ant visits in week 1–4 when compared with pretreatment data.

In the 2019 study, the three groups of houses showed similar levels of Argentine ant foraging activity (Kruskal–Wallis test: $P = 0.8$) before the initial spray treatments were applied. Pretreatment ant visit numbers for conventional #1, conventional #2, and IPM houses were 21,283 ± 9,407, 19,863 ± 8,234, and 21,433 ± 4,592 per monitoring vial (mean ± SEM), respectively.

Over the entire study period, the ant visit numbers in conventional group #1 showed minimal changes over time (Friedman test: $F = 3.07$, $P = 0.02$; Fig. 2A). Multiple comparison tests indicated that significant changes occurred between week 5 and 6 (reduction), and between week 6 and 8 (increase), during which no treatments were made. The number of ant visits in conventional group #2 showed no significant changes over time (Friedman test: $F = 0.36$, $P = 0.90$; Fig. 2B). During the entire study period, the untreated control house did not show any consistent drop in ant activity. For example, the untreated control house recorded 31,010 ant visits in pretreatment (mid-July), 57,780 in week 1 (86% increase when compared with pretreatment data), 23,759 in week 2 (23% decrease), 33,271 in week 4 (7% increase), 55,667 in week 7 (80% increase), and 12,289 in week 10 (60% decrease).

In contrast, ant visit numbers in the low-impact IPM group declined significantly over time (Friedman test: $F = 6.00$, $P = 0.0006$). Multiple comparison tests indicated that both the initial perimeter spray treatment (between pretreatment and week 1) and the follow-up treatment with the biodegradable hydrogel bait (between

Table 1. Treatment protocols used in the 2019 study

Treatment protocol	Conventional #1	Conventional #2	Low-impact IPM
Initial perimeter treatment	0.03% fipronil Perimeter (15 cm up and 15 cm out) 1 liter/linear 50 m (0.25 gal/160 linear ft) of diluted spray		0.03% fipronil + pheromone adjuvant
Follow-up maintenance treatment	0.06% bifenthrin 4 liters/100 m ² (1 gal/ 1,000 ft ²) of diluted spray	118 ml (4 ounces) of Essentria IC3 per gallon of water 8 liters/100 m ² (2 gal/1,000 ft ²) of diluted spray	Biodegradable hydrogel bait (1% boric acid) + pheromone adjuvant 4–8 liters/100 m ² (1–2 gal/1,000 ft ²)

week 4 and 5) provided significant reductions in the ant foraging activity level immediately after those treatments (Fig. 2C). By week 8, the houses in the IPM protocol had an overall 80% reduction in ant activity level when compared with pretreatment data.

Pesticide Use and Treatment Time

The overall amount of spray used per house for the initial perimeter treatment was 0.9–1.2 liters (0.23–0.31 gal) with all three protocols having a similar amount of fipronil applied per house. Time spent for the initial treatment was 5–8 min. For the follow-up treatment, the conventional protocol #1 had the smallest amount of material applied (1 liter per house) compared to the other protocols (3.8 and 5.6 liters per house for conventional #2 and IPM, respectively) (Table 2). Interestingly, the baiting in the IPM protocol had substantially shorter treatment time (7.4 min) than the other protocols (about 10 min).

Discussion

The 2017 study indicated that one-time boric acid hydrogel bait treatment provided an 87% reduction in ant activity by week 4 post-treatment. The boric acid hydrogel bait treatment took a few weeks to achieve acceptable levels (>80%) of Argentine ant control when used as a stand-alone method. Klotz et al. (1998) also noted that boric acid liquid baiting provided a significant reduction of Argentine ant foraging activity starting around week 4 post-treatment.

Data from conventional protocols #1 and 2 in the 2019 study indicated that the use of 0.03% fipronil alone for perimeter treatment failed to provide 4-wk control of Argentine ants. Large variations in ant foraging activity levels across different houses might be responsible, at least in part, for the overall nonsignificant reduction of ant activity at week 1 post-treatment. In both conventional protocols, two out of five houses had increased ant activity levels at week 1 when compared with corresponding pretreatment data. It is possible that the amount of fipronil applied was simply too low to be consistently effectual across different houses. Klotz et al. (2010) also reported that 1.9 liters (0.5 gal) of 0.06% fipronil perimeter application alone provided a widely variable control efficacy, with 38–75% reduction in Argentine ant activity by week 4. However, it is important to note that both the application rate and concentration of fipronil used by Klotz et al. (2010) were about twice as high as those employed in the current study. The current label of Termidor SC allows up to 4 separate applications per calendar year in California. However, it is not clear if additional applications of 0.03% fipronil spray would provide an acceptable level of control.

In contrast, the addition of the pheromone adjuvant in the fipronil spray reduced this large variation among different houses. All five houses in the low-impact IPM protocol had substantial reductions in ant foraging activity level at week 1, showing a statistically significant difference when compared with pretreatment data (65% reduction). The level of ant activity decreased until week 2 (85% reduction). The current findings corroborate the utility of pheromone adjuvant in improving control efficacy of a nonrepellent spray insecticide (Choe et al. 2014). The addition of pheromone will also reduce the amount of fipronil applied around structures to control ants and, thereby, reduce potential runoff.

By week 4, all treatment protocols (including the IPM protocol) experienced some levels of recovery in Argentine ant activity. Follow-up maintenance treatment with the bifenthrin spray alone (in conventional protocol #1) did not provide any significant reduction in ant foraging activity (four of five houses had increased ant

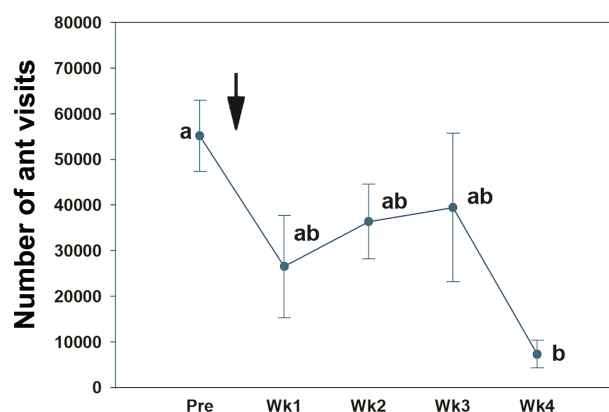


Fig. 1. Level of Argentine ant foraging activity (number of ant visits at the monitoring tubes; mean \pm SEM, $n = 4$) in the 2017 study. Arrows indicate the timing of a one-time boric acid hydrogel bait treatment. Data with different letters are significantly different (Friedman test followed by Conover all pairwise comparison test: $\alpha = 0.05$). Pre: pretreatment; Wk: week post-treatment.

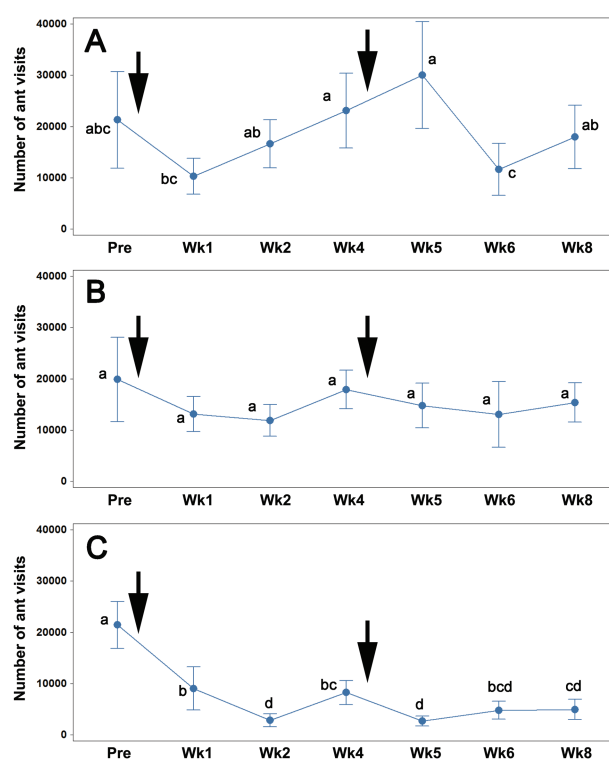


Fig. 2. Level of Argentine ant foraging activity (number of ant visits at the monitoring tubes; mean \pm SEM, $n = 5$ for each treatment protocol) for (A) conventional protocol #1, (B) conventional protocol #2, and (C) low-impact IPM protocol in the 2019 study. Arrows indicate the timing of initial perimeter spray treatment (left) and follow-up maintenance treatment (right). Data with different letters within a treatment are significantly different (Friedman test followed by Conover all pairwise comparison test: $\alpha = 0.05$). Pre: pretreatment; Wk: week post-treatment.

activity). This lack of efficacy might be due to the fast action or repellency of the pyrethroid insecticide sprays that would result in fewer ants being exposed and killed (Knight and Rust 1990, Tay and Lee 2015). Relatively low application rate and targeted use of bifenthrin spray in the current study (by following the restrictive use guidelines) may be also responsible for the outcome. For example, only

Table 2. Pesticide use, treatment time, and estimated costs associated with the three protocols applied around structures (average from five houses)

Treatment protocol	Conventional #1			Conventional #2			Low-impact IPM		
	Items	Quantity	Costs	Items	Quantity	Costs	Items	Quantity	Costs
Initial perimeter treatment	0.03% fipronil ^a	1.2 liters (0.31 gal)	\$0.37	0.03% fipronil	0.9 liter (0.23 gal)	\$0.28	0.03% fipronil	1 liter (0.25 gal)	\$0.46
	Labor	8 min	\$8.00	Labor	5 min	\$5.00	Pheromone adjuvant ^b	6.6 ml	\$0.66
							Labor ^c	7 min	\$7.00
Follow-up maintenance treatment	0.06% bifenthrin ^d	1 liter (0.26 gal)	\$0.31	Essential Oil ^e	3.8 liters (1 gal)	\$4.25	Boric acid hydrogels ^f	5.6 liters (1.48 gal)	\$5.60
	Labor	10 min	\$10.00	Labor	10.8 min	\$10.80	Pheromone adjuvant	5.6 ml	\$0.56
							Labor	7.4 min	\$7.40
Total cost/home			\$18.68			\$20.33			\$21.68

^aBased on the price of Termidor SC (20 oz) - \$70^bBased on estimated price \$0.1 for 1 ml (selected from \$0.085/ml - \$0.1/ml range)^cBased on estimated hourly rate \$60^dBased on the price of Talstar P Professional (32 oz) - \$40^eBased on the price of Essentria IC3 (32 oz) - \$34^fBased on estimate price \$1 for 1 L

pervious (e.g., soil, lawn) areas around the structure were treated with a band application (0.6 m or 2 ft width). All horizontal impervious surfaces (e.g., concrete) and other adjacent vegetated areas were treated only with 'spot' (0.19 m² or 2 ft² in size) or 'pin stream' (up to 2.54 cm or 1 inch wide) applications. Even though four of five houses showed some reductions in ant activity levels after the botanical insecticide spray application when compared with week 4 data (in conventional protocol #2), our data indicated that the botanical insecticide sprays alone failed to provide any significant reduction in ant foraging activity.

In contrast, 1% boric acid bait delivered with biodegradable hydrogels (in low-impact IPM protocol) provided consistent efficacy across all houses tested, keeping the ant activity levels low at week 5 (88% reduction). All five houses had reductions in the ant foraging activity level immediately after the baiting (week 5), showing a statistically significant difference when compared with week 4 data. The apparent discrepancy in the speed of action of boric acid baiting between 2017 and 2019 studies (i.e., 4 wk for 2017 and 1 wk for 2019) may be, in part, due to the relatively high pretreatment ant numbers in the 2017 study compared to the 2019 study (i.e., natural variations between different houses and sites). Also, the hydrogel bait application rate was substantially higher in the 2019 study compared to the 2017 study (i.e., 5.6 vs 3 liters per house).

If urban IPM programs are not economically feasible, PMPs are not going to incorporate them into their operations. We calculated the costs of the low-impact IPM strategy and the two conventional treatments (Table 2). The baiting in the IPM protocol had substantially shorter treatment time than the other protocols, indicating the ease of application of the hydrogel baits with the hand-held spreaders (Table 2). Since PMPs typically spend about 20 min treating a typical residential account for ants (Choe et al. 2019), the time component of tested protocols was considered reasonable. The cost of the low-impact strategy with pheromone adjuvant and boric acid hydrogel baits was comparable to that of conventional treatments. The low-impact strategy has an extra cost saving measure by eliminating the number of residences that need to be re-treated because of high numbers of ants.

The novel spray and hydrogel bait protocol developed in the current study was a cost-effective treatment providing season-long control for Argentine ants without repeated use of sprays. The pheromone adjuvant maximized the efficacy of the fipronil spray. When used as a stand-alone method, the biodegradable hydrogel bait with boric acid takes a few weeks to achieve acceptable levels of control (>80% reduction) for Argentine ants. Thus, perimeter treatment with fipronil and pheromone provided the initial quick control. With its relatively low toxicity on nontarget organisms, boric acid baiting was an important tool for the follow-up maintenance services. In the past, relatively high costs associated with materials and labor have been a drawback for conventional baiting methods that require bait stations. The use of an alginate hydrogel matrix as a carrier of liquid bait is an important breakthrough in addressing this challenge. The combination of a spreadable hydrogel bait and an effective perimeter treatment is an excellent and sustainable approach in developing Argentine ant IPM programs for PMPs.

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