# Final Report

Reporting Period: 10-19-2018 to 8-31-2020

Submittal Date: 10-21-2020

**Project Title:** **"Improving Urban Pest Ant Management by Low-Impact IPM Strategies"**

Grant Agreement No.: 26710

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**<u>Please see following pages for the contents of the final report (total 14 pages including this cover page).</u>**

Based on my inquiry of the persons who manage the project, or those directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete.

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## EXECUTIVE SUMMARY

Pest ants are among the top reasons for the public calling for professional pest management service. Residual treatment is often employed by professionals for outdoor treatment of pest ants. In an effort to minimize the environmental impacts of off-site movement of insecticides used in outdoor treatments, various reduced-risk strategies have been developed and implemented. However, it remains important for the reduced-risk strategies to maintain an acceptable level of control efficacy.

Two new technologies (spray with a pheromone adjuvant + biodegradable hydrogel bait delivery method) were used to develop a unique IPM protocol for Argentine ant at urban structural settings. The IPM protocol included a one-time perimeter treatment with 0.03% fipronil (mixed with a pheromone adjuvant) at the beginning of the ant season to achieve a quick knock down. The initial spray application was followed by hydrogel baiting with boric acid (1%) as a one-time supplementary or maintenance treatment. This low-impact IPM protocol was compared with other two 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. The protocols were compared for efficacy based on the Argentine ant foraging activity. Insecticide use information and service time were also recorded and compared among different treatment protocols.

Our research findings suggest that the pheromone adjuvant for perimeter spray and biodegradable hydrogel bait containing boric acid can be effective and feasible tools for Argentine ant IPM. Without the pheromone adjuvant, one-time application of 0.03% fipronil perimeter treatment following the California specific label instruction did not provide consistent control. However, the pheromone adjuvant maximized the efficacy of residual spray products. Pyrethroid and essential oil insecticide sprays did not provide consistent control of Argentine ants when used for follow-up maintenance. With its relatively low toxicity profile on non-target organisms, boric acid baiting is an important tool for the follow-up maintenance services. Relatively high cost associated with material and labor has been a drawback for conventional baiting methods. The use of a biodegradable hydrogel matrix as a carrier of liquid bait can be an important breakthrough in addressing this challenge.

## INTRODUCTION

In many urban residential areas of the United States, the Argentine ant is one of the most common nuisance ant species treated by pest management professionals (PMPs). Contact and residual insecticide sprays are among the most common treatment options for Argentine ant control because of their ease of application and cost-effectiveness. However, many of these insecticides are frequently detected in urban waterways (Greenberg et al., 2014, references cited therein).

In this study, we used two new approaches (i.e., pheromone adjuvant for spray applications and biodegradable hydrogel bait) to develop a low-impact IPM protocol (Choe et al., 2014; Choe and Campbell, 2014; Tay et al., 2017). It was compared with other two other methods that mimic the treatment protocols that are often adopted by PMPs. A one-time perimeter treatment with a fipronil spray at the beginning summer was incorporated in all 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. Insecticide use amount and treatment time data were also compared between different treatment protocols.

## MATERIALS AND METHODS

### Experimental settings

Residential houses in Riverside, CA, USA were used for the experiments. Five houses were assigned to each of three protocols, each house representing one replicate. Foraging activity level of ants was estimated based on the total amount of sucrose solution consumed over a 24-hour period (Welzel et al., 2016). The average value from 10 monitoring sites around foundation was used for statistical analyses. To understand the overall Argentine ant activity in the absence of treatment efforts, an untreated control house was monitored over the entire project period.

### Conventional protocols

Two different conventional protocols 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) at early summer (Fig. 1), followed by maintenance treatment with another spray product (Table 1). For the maintenance treatment, conventional protocol #1 used a 0.06% bifenthrin spray (Talstar P, FMC, 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 (Fig. 2). 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 or early September (week 4).

**Low-impact IPM protocol**

The low-impact IPM protocol consisted of a one-time fipronil spray treatment (mixed with a pheromone adjuvant – microencapsulated (Z)-9-hexadecenal, Suterra, LLC., Bend, OR; 25 ml per 3.8 liter of spray) at early summer followed by the use of a biodegradable hydrogel bait (1% boric acid) at week 4 post-treatment as a maintenance treatment (Table 1).

The biodegradable hydrogel bait was produced by the method described by Tay et al. (2017) with minor modifications. Several methodological modifications were incorporated in the method to either establish or achieve following:

a) Three-step and three-day manufacturing process. The first step (day 1) is the preparation of the alginate solution (1% alginate). The second step (day 2) is the formation of the hydrogels with an appropriate cross-linking time using a calcium chloride solution (0.5% $CaCl_2$). The final step (day 2 – day 3, overnight) is the conditioning of the hydrogel to create hydrogel beads containing 25% sucrose and 0.5% boric acid (wt/vol).
b) Quick production of the hydrogel beads (e.g., 1 -2 kg of hydrogel in 5 min) for conditioning. The conditioning process takes about 18 h. In the final hydrogel product, each bead contained 0.14-0.17 ml of the liquid bait (Fig. 3).
c) Precise concentrations of sugar (25%) and boric acid (0.5 %) in the final hydrogel bait.

d) Ease of application with hand-held spreader. (Fig. 4).

e) Potential rehydration of the hydrogel if there is enough amount of moisture provided (Fig. 5).

The Na-Alg solution (1%) was slowly dispensed dropwise through a modified 8-inch shower head nozzles (1.6 mm diameter). The droplets were immediately collected in a plastic container with 0.5% $CaCl_2$ crosslinker solution. After 2 minutes, the resulting hydrogel beads were filtered out from the crosslinking solution and rinsed with clean water. The rinsed hydrogel beads were "conditioned" by submerging them in a liquid bait containing sucrose and boric acid overnight (24 h). Concentrations of the sucrose and boric acid in the final hydrogel bait were 25 and 1%, respectively. To improve stability of the final hydrogel bait, 0.25% sorbic acid potassium salt was incorporated in the final hydrogel bait. A pheromone adjuvant (microencapsulated (Z)-9-hexadecenal; 1 ml per liter of bait) was also mixed with the hydrogel bait immediately before application.

About 4-7 liter of hydrogel bait was used per house (approximately 40-70 g boric acid per house). 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 (Fig. 6). As in the conventional protocols, the bait was not used on horizontal impervious surfaces (e.g., concrete).

**Data collection and statistical analyses**

For the initial treatment, the sites were monitored on day 1 pre-treatment, and weeks 1, 2, and 4 after the treatment. Follow-up maintenance treatment was made after the monitoring at week 4, and sites were further monitored at weeks 5, 6, and 8. For each treatment, the amount of spray and bait applied (in liter) and the time required to make the applications were recorded.

A Kruskal-Wallis test was used to compare three groups of houses in their pre-treatment ant activity levels. A Friedman test, a non-parametric alternative to a one-way repeated-measures ANOVA (Kim, 2014), was used to assess differences in ant visits between different monitoring time points within a treatment protocol. If the Friedman test indicated a significant difference among different monitoring time points, Conover's all-pairwise comparisons test was used for multiple comparisons (Analytical Software, 2008).

## RESULTS AND DISCUSSION

### Control efficacy

Before the initial spray treatment, three groups of houses showed similar levels of Argentine ant foraging activity (Kruskal-Wallis test: $P = 0.8$). Pre-treatment ant visit numbers for conventional #1, conventional #2, and IPM houses were 21,283 ± 21,034, 19,863 ± 18,413, and 21,433 ± 10,268 per monitoring vial (mean ± SD), respectively.

Over the entire study period, the ant visit numbers in conventional #1 group showed some significant changes over time (Friedman test: $F = 3.07$, $P = 0.02$) (Fig. 7A). However, multiple comparisons test 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 numbers of ant visit in conventional #2 group showed no significant changes over time (Friedman test: $F = 0.36$, $P = 0.90$) (Fig. 7B). During the entire study period, the untreated control house did not show any consistent drop in ant activity level.

In contrast, ant visit numbers in the reduced-risk IPM group showed significant changes over time (Friedman test: $F = 6.00$, $P = 0.0006$). Multiple comparisons test indicated that both the initial perimeter spray treatment (between pre-treatment and week 1) and the follow-up treatment with biodegradable hydrogel bait (between week 4 and 5) provided significant reductions in the ant foraging activity level immediately after those treatments (Fig. 7C).

### Pesticide use and treatment time

The pesticide use and treatment time data are shown in Table 2. The overall amount of spray used per house for the initial perimeter treatment was 0.9-1.2 liter (0.23-0.31 gallon), providing all three protocols had similar amount of fipronil applied per house. Time spent for the initial treatment was 5-8 minutes. 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 liter per house for conventional #2 and IPM, respectively). Relatively low application rate and targeted use of bifenthrin spray in the current study may be responsible for this difference. For example, only 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 $m^2$ or 2 $ft^2$ in size) or "pin stream" (up to 2.54 cm or 1 inch wide) applications. Interestingly, in spite of the largest amount of material being applied, the baiting in the IPM protocol had substantially shorter treatment time (about 7 minutes) than the other protocols (about 10 minutes), indicating the ease of application of the hydrogel baits with the hand-held spreaders. Since PMPs spend about 20 minutes treating a typical residential account for ants (Choe et al., 2019), the time component of tested protocols was considered reasonable.

## CONCLUSIONS

Data from conventional protocols #1 and 2 indicated that the use of 0.03% fipronil alone for perimeter treatment failed to provide 4-weeks control of Argentine ants. Large amounts of variation in ant foraging activity levels across different houses might be responsible, at least in part, for the overall non-significant reduction of ant activity at week 1 post-treatment. For example, in both conventional protocols, two of five houses had increased ant activity levels at week 1 when compared to corresponding pre-treatment data. Additional applications of fipronil spray might be necessary to provide an acceptable level of control. The current label of Termidor SC allows up to 4 separate applications per calendar year in California.

In contrast, the addition of the pheromone adjuvant in the fipronil spray reduced this large variation among different houses. All five houses in the reduced-risk IPM protocol had substantial reductions in ant foraging activity level at week 1, showing a statically significant difference when compared to pre-treatment 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 non-repellent spray insecticide (Choe et al., 2014).

By week 4, all treatment protocols (including IPM protocol) experienced some levels of recovery in Argentine ant activity. Follow-up maintenance treatment with the bifenthrin spray alone did not provide any significant reduction in ant foraging activity (4 of 5 houses had increased ant activity). Even though 4 of 5 houses showed some reductions in ant activity levels after the botanical insecticide spray application when compared to week 4 data, 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 in biodegradable hydrogels provided a consistent efficacy across all houses tested, keeping the ant activity levels low at week 5 (88% reduction). All five houses had reductions in ant foraging activity level immediately after the baiting (week 5), showing a statistically significant difference when compared to week 4 data. By week 8, the houses in the IPM protocol had an overall 80% reduction in ant activity level when compared to pre-treatment data.

The novel spray and bait protocol developed in the current study was effective in providing a season-long control for Argentine ants without repeated use of sprays. The pheromone adjuvant will maximize the efficacy of residual spray products. When used as a stand-alone method, the biodegradable hydrogel bait with boric acid takes a few weeks to achieve the acceptable levels of control (>80% reduction) for Argentine ants (D.-H. Choe, unpublished data). Thus, perimeter treatment with an effective spray material was useful in providing the initial quick control. With its relatively low toxicity profile on non-target organisms, boric acid baiting is an important tool for the follow-up maintenance services. Relatively high cost associated with material and labor has been a drawback for conventional baiting methods. The use of a biodegradable hydrogel matrix as a carrier of liquid bait is an important breakthrough in addressing this challenge.

## ACKNOWLEDGMENTS

We thank Kathleen Campbell, Ho Eun Park, Les Greenberg, Michael K. Rust, Qian Yue Lu, Benning Le, and Maddux Le for their assistance in the project. The pheromone adjuvant used in the current study was provided by Suterra, LLC. Funding for the study was provided by California Department of Consumer Affairs, Structural Pest Control Board (grant agreement #26710).

## REFERENCES CITED

**Analytical Software. 2008.** Statistix 9 user's manual. Analytical Software, Tallahassee, FL.

**Choe, D.-H, K. Tsai, C. Lopez, K. Campbell. 2014.** Pheromone-assisted techniques to improve the efficacy of insecticide sprays against *Linepithema humile* (Hymenoptera: Formicidae). J. Econ. Entomol. 107: 319-325.

**Choe, D.-H., K. Campbell. 2014.** Fatal attraction - the application of lure-and-kill tactics for urban pest ants. Pest Control Technology. 42: 44, 48, 50-51, 54, 56, 58.

**Choe, D.-H., E. Paysen, L. Greenberg, K. Campbell, and M. Rust. 2019.** A closer look: Argentine ant control. Pest Control Technology. 47: 130-132, 134, 135.

**Greenberg, L., M. K. Rust, J. Richards, X. Wu, J. Kabashima, C. Wilen, J. Gan, and D.-H. Choe. 2014.** Practical pest management strategies to reduce pesticide runoff for Argentine ant (Hymenoptera: Formicidae) control. J. Econ. Entomol. 107: 2147-2153.

**Kim, H.-Y. 2014.** Statistical notes for clinical researchers: Nonparametric statistical methods: 2. Nonparametric methods for comparing three or more groups and repeated measures. Restor. Dent. Endod. 39: 329-332.

**Welzel, K. F., D.-H. Choe. 2016.** Development of a pheromone-assisted baiting technique for Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 109: 1303-1309.

**Table 1.** Treatment protocols used in the current study

<table>
<tr><th>Treatment protocol</th><th>Conventional #1</th><th>Conventional #2</th><th>Reduced-risk IPM</th></tr>
<tr><td>Initial perimeter treatment</td><td colspan="2">0.03% fipronil<br><br>Perimeter (15 cm up and 15 cm out)<br><br>1 L / linear 50 m (0.25 gal / 160 linear ft) of diluted spray</td><td>0.03% fipronil<br><br>+<br><br>pheromone adjuvant</td></tr>
<tr><td>Follow-up maintenance treatment</td><td>0.06% bifenthrin<br><br>4 L / 100 $m^2$ (1 gal / 1,000 $ft^2$) of diluted spray</td><td>118 ml (4 ounces) of Essentria IC3 per 3.8 L (1 gal) of water<br><br>8 L / 100 $m^2$ (2 gal / 1,000 $ft^2$) of diluted spray</td><td>Biodegradable hydrogel bait (1% boric acid) + pheromone adjuvant<br><br>4-8 L / 100 $m^2$ (1-2 gal / 1,000 $ft^2$)</td></tr>
</table>

**Table 2.** Pesticide use amount and the time required to treat each house (average value from five houses)

| Treatment protocol | Conventional #1 | Conventional #2 | Reduced-risk IPM |
|---|---|---|---|
| Initial perimeter treatment | 1.2 L (0.31 gal)<br><br>8 min | 0.9 L (0.23 gal)<br><br>5 min | 1.0 L (0.25 gal)<br><br>7 min |
| Follow-up maintenance treatment | 1.0 L (0.26 gal)<br><br>10 min | 3.8 L (1 gal)<br><br>10.8 min | 5.6 L (1.48 gal)<br><br>7.4 min |

**Fig. 1.** Treatment of a house with a perimeter spray (fipronil spray).

**Fig. 2.** Treatment of a house with a spot treatment (bifenthrin or botanical spray).

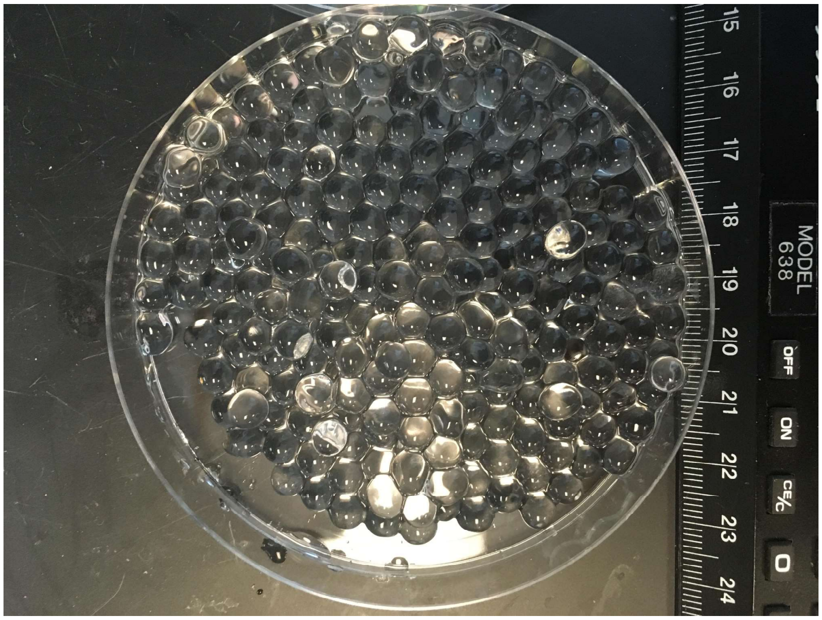


**Fig. 3.** Final hydrogel baits manufactured using the modified methods.

**Fig. 4.** Testing with the hand-held spreader.

**Fig. 5.** Rehydration test with the final hydrogel bait. The hydrogel bait beads on the left are completely dried. When enough amount of water is provided, these dry hydrogel beads can be rehydrated (right), becoming palatable to forging ants once again.

**Fig. 6.** Treatment of a house with biodegradable hydrogel beads containing 25% sucrose and 1% boric acid.

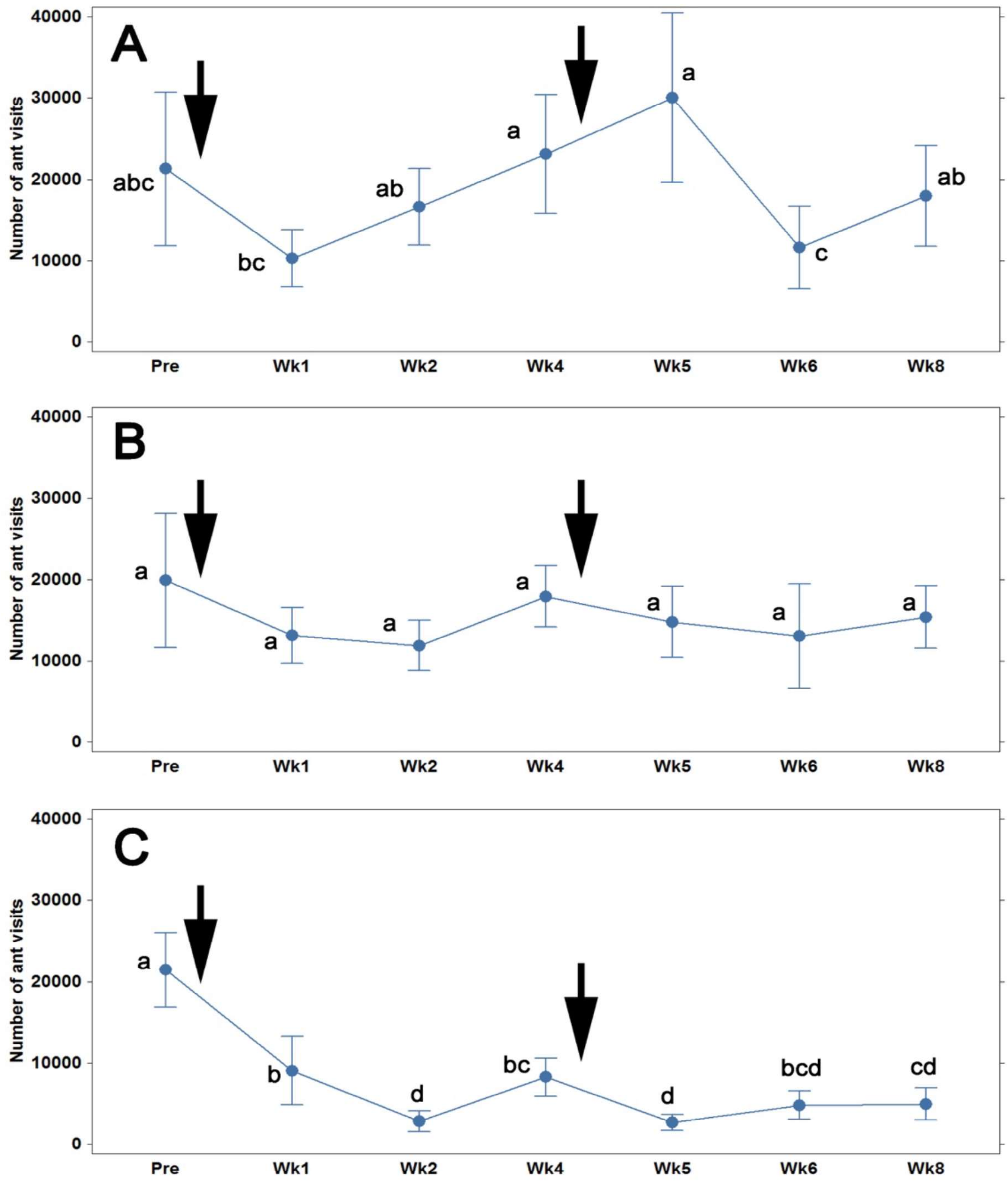


**Fig. 7**. Level of Argentine ant foraging activity (number of ant visits at the monitoring tubes; mean ± SEM, n = 5 for each treatment protocol) for (A) conventional protocol #1, (B) conventional protocol #2, and (C) low-risk IPM protocol. Arrows indicate the timing of initial perimeter spray treatment (left) and follow-up maintenance treatment (right). Data with different letters within a treatment are significantly different (Conover's all pairwise comparison test followed by Friedman's test: α = 0.05). Pre: pre-treatment; Wk: week post-treatment.