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Development of a Pheromone-Assisted Baiting Technique for Argentine Ants (Hymenoptera: Formicidae)

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Abstract

Current control measures for Argentine ants, *Linepithema humile* (Mayr), in urban settings typically include perimeter applications of insecticides around structures, resulting in potential problems with insecticide runoff and environmental contamination. Insecticidal baits can be an effective alternative to perimeter spray applications and are largely considered target-specific with minimal nontarget impact and environmental contamination. We report a "pheromone-assisted baiting technique" as an economically viable approach to maximize the efficacy of conventional baits targeting Argentine ants. Laboratory experiments with a commercially available gel bait indicated that foraging activity and final mortality of Argentine ants were significantly improved by incorporating (Z)-9-hexadecenal in the bait. The field study demonstrated that the pheromone-treated gel bait achieved a 74% reduction in Argentine ant activity by the end of 4 wk when it was compared with its own pretreatment value. This was a significant improvement over the untreated gel bait that provided a 42% reduction over the same period of time. The pheromone-assisted baiting technique has the potential in providing effective ant control with reduced amount of insecticides applied in the environment.

Key words: *Linepithema humile*, ant bait, pheromone, (Z)-9-hexadecenal

The invasive Argentine ant, *Linepithema humile* (Mayr), has become one of the major nuisance pests in many urban areas in the United States over the past several decades (Silverman and Brightwell 2008, Gilboa et al. 2012). It is the dominant urban ant pest in California and Georgia and a significant localized pest in the other southeastern states such as South Carolina, Alabama, Mississippi, Louisiana, Florida, Tennessee, and North Carolina (Bambara et al. 2006). For instance, one survey indicated that 85% of urban pest control services are allocated solely to the control of Argentine ants in California (Field et al. 2007). According to another survey conducted in North Carolina in 2000, Argentine ants were present in >20% of the samples collected by the local pest management professionals (PMPs) from urban areas (Bambara et al. 2006).

Residual insecticide sprays are widely used to control the Argentine ant in urban settings. Their simple application and effectiveness in providing rapid control make them a popular choice among PMPs (Rust et al. 2003, Klotz et al. 2008a). Pyrethroids (e.g., bifenthrin, cyfluthrin, cypermethrin, and permethrin) and phenylpyrazole (e.g., fipronil) are among the most common active ingredients that are used in these residual insecticide sprays (Rust 2001, Rust et al. 2003, Silverman and Brightwell 2008). Based on 2012 data, 25,445 kg of bifenthrin (active ingredient) and 24,166 kg of fipronil (active ingredient) were formulated into various spray products used by licensed PMPs for urban pest management in California (California Department of Pesticide Regulation [CDPR] 2012). Consequently, some of these active ingredients, and their toxic degradation products, are frequently detected in urban waterways and aquatic sediments (Weston et al. 2009, Lao et al. 2010, Delgado-Moreno et al. 2011).

Frequent detection of these active ingredients in urban water ways is concerning because of the potential effects on nontarget species (Bonmatin et al. 2015). For example, bifenthrin was identified as the primary causative agent of toxicity to an amphipod indicator species, *Hyalella azteca*, with additional toxicity being attributed to the presence of cyfluthrin and cypermethrin (Weston et al. 2005). *Hyalella azteca* is an aquatic grazer that is often used to determine the nontarget effects of pesticide contaminants in aquatic systems (Weston et al. 2005). Additionally, fipronil was detected in urban waterways in amounts that exceeded the LC_{50} values for many indicator arthropod species such as mysid shrimp and grass shrimp (Gan et al. 2012). Developing new ant control strategies that are not solely dependent upon residual sprays will help mitigate the negative impact on water quality.

Baiting is an effective alternative to insecticide sprays for managing pest ants (Klotz et al. 2003). When properly designed and used, insecticidal baits can provide several potential advantages over the insecticide sprays. For example, baits can be designed to achieve target specificity and minimal off-site movement of insecticidal active ingredients (Klotz et al. 2010). However, for a bait to be acceptable as an effective tool for a practical ant pest management program, the baits must meet several requirements. For example, the toxic baits should maintain palatability, nonrepellency, delayed toxicity, and transferability over the baiting period (Silverman and Roulston 2001). Maintaining these bait characteristics depends on not only the chemical constituents (i.e., active and inactive ingredients) of the baits but also their concentrations. Additionally, baits should restrict access of nontarget organisms while eliminating or reducing the target ant populations (Silverman and Brightwell 2008).

One of the potential ways to improve current baiting technologies is to use insect pheromones in conjunction with existing bait products (Vander Meer 1996). By incorporating species-specific insect pheromones to the bait matrix, the bait can be designed to be quickly discovered and consumed by the desired pest species before any detrimental changes of the bait matrix or active ingredient(s) occurs. In particular, fermentation and increased viscosity can quickly occur for the sugar-containing liquid or gel baits placed under warm and dry conditions, negatively impacting the palatability of the baits for liquid-feeding ants (Cooper et al. 2008, Choe et al. 2010). Several studies have explored the possibility of using a synthetic trail-following pheromone to develop practical management strategies for the Argentine ant. Choe et al. (2014) reported that the addition of (Z)-9-hexadecenal, a putative trail pheromone component of Argentine ants, into insecticide sprays improved the sprays efficacy because (Z)-9-hexadecenal attracts worker ants from nearby locations to the insecticide spray deposits. Greenberg and Klotz (2000) showed that (Z)-9-hexadecenal, when mixed with a sugar solution, increases Argentine ant consumption. However, this study did not determine if the efficacy of insecticidal bait can be improved by incorporating the pheromone into the bait.

In this study, we explored if the incorporation of Argentine ant pheromone, (Z)-9-hexadecenal, could improve the efficacy of existing gel baits. We used a gel bait product that is broadly marketed throughout California (CDPR 2012) and other parts of the United States for residential ant control. Laboratory studies were conducted to determine if the addition of (Z)-9-hexadecenal into a gel bait could increase the Argentine ants' foraging activity on that gel bait, and thus, increase the resulting mortality. A field study was also conducted to determine if the addition of (Z)-9-hexadecenal into that same gel bait could increase the bait consumption and consequently, provide improved Argentine ant control in the urban residential settings.

Materials and Methods

Ants

Argentine ants were collected from the biological control grove on the University of California, Riverside campus. Ant nests were excavated from the ground and transported to the laboratory, where the ants were extracted from the soil (Hooper-Bui and Rust 2000). Laboratory stock colonies were maintained in plastic boxes (26.5 by 30 by 10 cm^3); the inner sides were coated with Teflon (Fluoropolymer resin, type 30, DuPont Polymers, Wilmington, DE) to prevent the ants from escaping. Each colony was provided with two or three artificial nests constructed from plaster-filled Petri dishes (9 cm diameter by 1.5 cm depth) with a smaller cylindrical area (5 cm diameter by 1 cm depth) in the center of the dish, to serve as a nesting space. The colonies had free access to fresh water and 25% (wt/vol) sucrose solution. Freshly killed American cockroaches, *Periplaneta americana* (L.), were provided three times a week as a protein source.

Insecticide

Optigard ant gel bait (Syngenta Crop Protection, Inc. Greensboro, NC) containing 0.01% (wt/wt) thiamethoxam as the active ingredient was used in the study. The gel bait was chosen based on its effectiveness in preliminary laboratory trials (K.F.W., unpublished data) and its common use by PMPs in California. Thiamethoxam requires consumption by the ants to become most effective. Additionally, the thiamethoxam ingested by one individual could be transferred to other individuals within the population via oral food exchange (i.e., stomodeal trophallaxis; Rust et al. 2004).

Laboratory Study

Laboratory studies were conducted to determine if the addition of (Z)-9-hexadecenal (Bedoukian Research, Inc., Danbury, CT) into a gel bait could increase the Argentine ants' short-term feeding activity on that gel bait, and thus, increase the resulting mortality. A group of Argentine ant workers (0.5 g), reared from laboratory stock colonies (see earlier text), was aspirated, anesthetized with CO_2, and placed in an empty plastic box arena (20 by 35 cm^2). The inner sides of the arena box were coated with Teflon (Fluoropolymer resin, type 30, DuPont Polymers) to prevent the ants from escaping. This method of preparation resulted in relatively similar-sized experimental colonies (745 ± 28 ants [mean ± SEM], $n = 36$, range 714–901). One reproductive (queen) was added to each of the experimental colonies. Each experimental colony was provided with one nest tube, constructed from a 50-ml Falcon plastic tube (Fisher Scientific, Waltham, MA) that was filled with 15 ml water and stopped with a cotton ball, and one sugar water dish containing 25% sucrose solution. The nest tube was placed in one end of the box, and the sugar water dish was placed next to the nest.

We compared the following gel bait treatments gel bait with pheromone (treatment gel bait) and gel bait only (standard gel bait). In addition to these two gel bait treatments, we also included a control group that only received 25% sucrose solution without any toxicant (untreated control), to estimate the natural mortality of laboratory colonies of Argentine ants. The gel bait was provided in a small plastic dish (cap of 1.5-ml microcentrifuge tube). For the treatment gel bait, 10 µl of (Z)-9-hexadecenal preparation, dissolved in acetone (10 µg/ml), was applied in the center of the dish. The solvent was allowed to evaporate for 1 min before placing 1 g of gel bait in the dish. Then, the gel bait was gently stirred with a stick, mixing the gel bait with the pheromone residue. The approximate final concentration of the pheromone in the gel bait was 0.1 µg/g. For the standard gel bait, 10 µl of clean acetone was applied to the dish before placing 1 g of gel bait. The standard gel bait was also stirred in a manner similar to the preparation of treatment gel bait. The bait dish containing either the standard or treatment gel bait was then placed in the plastic box arena at the opposite end from the nest tube, ~20 cm away from the nest entrance. Each experimental colony was randomly assigned to treatment gel bait, standard gel bait, or untreated control. Two gel bait treatments and the untreated control were replicated 12 times. The experimental colonies were provided with 25% (wt/vol) sucrose solution throughout the entire experimental period.

For the gel bait treatments, starting immediately after the introduction of the gel bait dish, each experimental colony was photographed every 3 min to accurately record the number of ants feeding on the gel bait over time. Photographs were taken for a total period of 30 min, resulting in 10 observations per trial. After 30 min, the bait dish was removed from the plastic box arena. The timed removal of the bait from the experimental colony was necessary because the continued presence of the gel bait in the colony box would rapidly satiate the experimental colonies contained in a small space (i.e., 700 cm^2), limiting the potential differentiation between treatment and standard gel baits (K.F.W., unpublished data). All experimental colonies were maintained at 23–25°C and 34–45% relative humidity for 7 d. Dead ants were removed daily from each box, and total cumulative number of the dead ants was counted on day 7.

Field Study

Field experiments were conducted to determine if the addition of (Z)-9-hexadecenal into the gel bait could increase the gel bait consumption over a long term (i.e., 4 wk). Control efficacies were also compared between treatment gel bait (gel bait with pheromone) and standard gel bait (gel bait only). The study was conducted in Riverside, CA, using residential houses that local homeowners voluntarily provided as research sites. Because the main focus of current study was examining the efficacy difference between treatment gel bait (with pheromone) and standard gel bait (without pheromone), we did not include untreated control houses. Additionally, it was impossible to find homeowners that were willing to cooperate in a study like this without receiving some kind of ant control measures.

Before the start of the experiment, houses were monitored for overall ant activity levels based on number of ant visits at monitoring tubes. The 15-ml Falcon plastic tubes (Fisher Scientific) containing 12 ml of 25% sucrose solution were used as monitoring tubes. For each house, 10 monitoring tubes were placed along the perimeter, and the other 10 tubes were placed about 3 m away from the house perimeter. After 24 h, the tubes were capped to prevent liquid loss during transportation, returned to the laboratory, and weighed. The weight data were corrected for evaporative loss of the liquid during the 24-h monitoring period (Klotz et al. 2008b). By calculating the difference between the initial weight of the sucrose solution placed into the monitoring tube and the adjusted weight of the remaining sucrose solution, we obtained the amount of sucrose solution consumed by the foraging ants over the 24-h monitoring period. A single Argentine ant typically consumes 0.0003 g of sucrose solution per visit; therefore, we assumed a direct relationship between amount of sucrose solution consumed and number of ant visits at the monitoring tube (Reierson et al. 1998). Based on this assumption, the number of ant visits per tube was estimated by dividing the consumption (g) by 0.0003 (g per visit). One advantage of this type of monitoring is it reflects an overall foraging activity of the ants over a relatively long period of time (i.e., 24 h) rather than estimating the ant activity based on a snapshot observation (e.g., counting ant numbers on a trail for a fixed amount time), in which the estimations could vary greatly depending upon the times of the day or ambient weather conditions. Based on preliminary monitoring, 10 houses with significant levels of Argentine ant activity (i.e., a minimum of total 84 ml loss from the 240 ml originally distributed in 20 monitoring tubes) were selected for this study.

Five of the 10 houses were treated with treatment gel bait, and the other five houses were treated with standard gel bait. Bait stations were constructed from graduated 15-ml Falcon plastic tubes (Fisher Scientific) with a small hole (3 mm diameter) drilled in the cap. The small entry hole in the cap allowed the foraging ants to access the gel bait inside of the bait station, while preventing nontarget organisms and water (from irrigation and natural precipitation) from entering into the bait station. Each bait station was loaded with 2 ml of thiamethoxam gel bait. For the treatment gel bait, 0.2 µg of (Z)-9-hexadecenal dissolved in 20 µl of acetone was applied on the top of the gel bait inside the tube and mixed by gently agitating the bait station. The standard gel bait consisted of thiamethoxam gel bait dispensed in the tube without any modification. Application of acetone on the bait did not alter normal foraging behavior of ants and overall mortality rates, compared with the standard gel bait (K.F.W., unpublished data).

Twenty bait stations were evenly distributed around each house, 10 along the perimeter of the house and 10 in the yard. We also referred to product label instructions for the placement of the gel bait. Each bait station was labeled and buried with only the entry hole exposed to soil surface. For the locations where bait stations could not be buried (e.g., hardscapes like the concrete patio), the tubes were laid on the ground in the notch of a small Lincoln Log (K'NEX Industries Inc., Hatfield, PA) and covered with plastic flower pots (15.5 cm diameter by 11.5 cm height) to protect it from sprinkler irrigation, pets, and direct sunlight. The bait stations were checked once a week for 4 wk. The amount of bait reduction per bait station was estimated by reading the 0.1-ml measured increments on the side of the bait stations. Stations that showed a substantial amount of reduction (i.e., >50% in volume) were replaced with new bait stations.

To determine the control efficacy, the activity level of Argentine ants was estimated using the monitoring tubes at weeks 1, 2, and 4 posttreatment. The bait stations were removed before placing monitoring tubes. Twenty monitoring tubes, 10 near the house and 10 away from the house, were placed in similar locations to where the bait stations had previously been located. After 24 h, the monitoring tubes were sealed and returned to the laboratory for weight measurement to estimate the number of ant visits (see earlier text).

Statistical Analyses

All statistical analyses were performed in R version 3.0.3 (R Development Core Team 2008). For the laboratory foraging study, the count data from 10 consecutive observations were averaged for each trial, and the average values were compared between treatment gel bait and standard gel bait only with a Welch two-sample *t*-test (R Development Core Team 2008). For the laboratory mortality data, a one-way analysis of variance (ANOVA) followed by a Tukey's honest significant test (HSD) test (R Development Core Team 2008) was used to compare the total cumulative numbers of dead ants at day 7 for both treatments and untreated control.

For the field study, amounts of gel bait consumed (ml) were compared between treatment and standard gel baits for each week of the experiment using a repeated measures ANOVA, with the fixed effect of treatment (treatment vs. standard gel baits) and a repeated effect of week. Average values from the 20 bait stations were used as data. A generalized mixed model (GLMM) was used to compare ant activity levels. The model contained fixed effects for week (pretreatment and weeks 1, 2, and 4 posttreatment) and treatment (treatment gel bait and standard gel bait). The interaction of week and treatment was also included as was a random effect term for "house." GLMM was performed using the "lmer" function from the lme4 package (Bates et al. 2014), and the "Anova" function of the package "car" was used to generate type II tests (Fox and Weisberg 2011). The levels of ant activity from the number of ant visits were compared between the treatment and standard gel bait houses for each week using a Wilcoxon rank sum test.

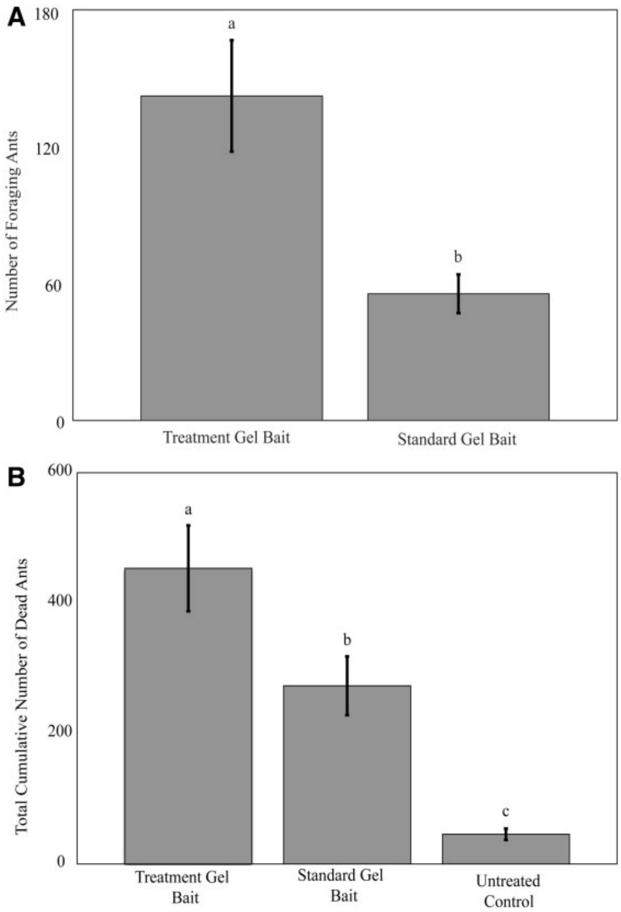

Fig. 1. Laboratory data results. (**A**) Number of foraging ants for treatment and standard gel baits. The height of each bar represents the number of foraging ants (mean ± SEM). (**B**) The total cumulative number of dead ants for treatment gel bait, standard gel bait, and untreated control after day 7. The height of each bar represents the total cumulative number of dead ants after day 7 (mean ± SEM). For each treatment, means with different letters are significantly different. See text for details of statistical analyses.

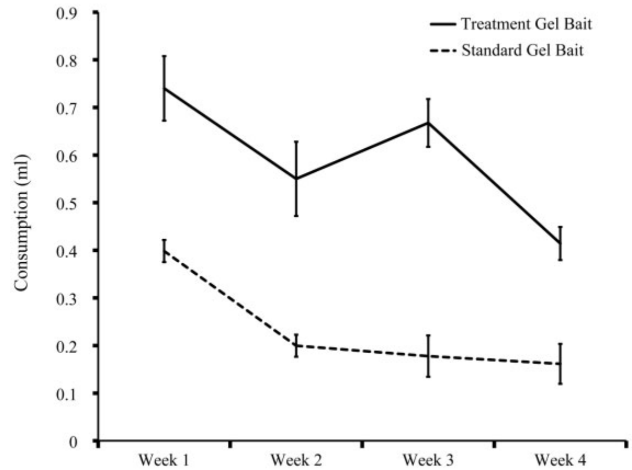

Fig. 2. Field study results showing amount of consumption (ml) of treatment and standard gel baits. Each point on the line graph represents the amount of consumption (mean ± SEM) for each week. See text for the details of statistical analyses.

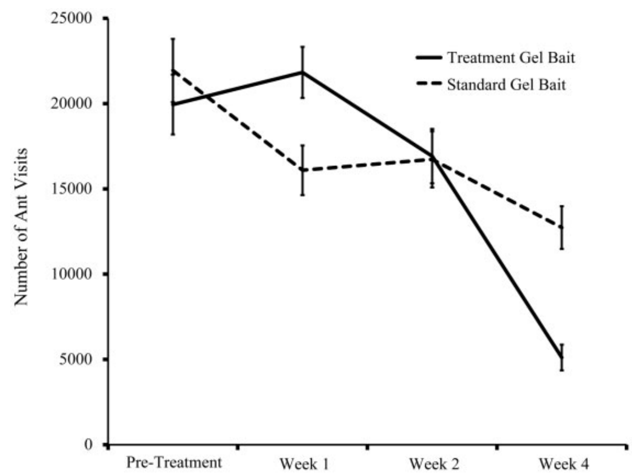

Fig. 3. Field study results showing the comparison between treatment and standard gel baits on the ant activity level. Each point on the line graph represents the number of ant visits (mean ± SEM) for each of the 24-h monitoring periods (i.e., pretreatment and week 1, 2, and 4 posttreatment). See text for the details of statistical analyses.

Results

Laboratory Study

Foraging activity of the Argentine ant was significantly greater in the treatment gel bait when compared with standard gel bait (Fig. 1A). On average, the treatment gel bait had twice as many foragers compared with the standard gel bait (142.3 ± 24.4 and 55.5 ± 8.5 [mean ± SEM] ants for the treatment and standard gel baits, respectively) over 30-min observation period ($T = 3.8$, df = 22, $P < 0.001$). The ANOVA with day 7 total cumulative mortality indicated significant variation among the three treatments ($F = 42.3$; df = 2, 4; $P < 0.001$; Fig. 1B). Day 7 mortality was significantly greater in the treatment gel bait (453.4 ± 65.8 ants) compared with standard gel bait (273.3 ± 44.9 ants; Tukey's HSD: $P < 0.001$). Day 7 total cumulative mortality from the untreated control (45.6 ± 8.6) was low, significantly different from both gel bait treatments (Tukey's HSD: $P < 0.001$).

Field Study

The average amount of gel bait consumption was significantly higher for treatment gel bait than for standard gel bait in posttreatment monitoring trials ($F = 98.9$; df = 1, 35; $P < 0.001$; Fig. 2). The number of ant visits at monitoring tubes varied significantly among weeks (GLMM: $\chi^2 = 74.5$; df = 3, 7; $P < 0.001$), but the main effect of treatments (treatment gel bait vs. standard gel bait) on averaged ant visits was not significant, indicating treatment alone did not influence overall ant visits ($\chi^2 = 0.82$; df = 1, 7; $P = 0.37$). However, when the interaction of the fixed effect of week and treatments was considered, there was a significant effect ($\chi^2 = 20.2$; df = 3, 7; $P < 0.001$; Fig. 3).

The levels of ant activity estimated from the number of ant visits were compared between the treatment and standard gel bait houses for each week (Fig. 3). The Wilcoxon rank sum test indicated that the houses assigned for treatment and standard gel bait applications were similar in Argentine ant activity for the pretreatment week (19,945 ± 1,757 vs. 21,939 ± 1,848 [mean ± SEM] ant visits for the treatment and standard gel bait houses, respectively; $Z = 0.4$, $P > 0.5$). In week 1, ant activity level of the treatment gel bait houses increased to 21,823 ± 1,498 ant visits, while that of the standard gel bait houses decreased to 16,091 ± 1,456 ant visits. Consequently, the houses treated with treatment gel bait had significantly higher ant activity compared with the houses treated with standard gel bait by the end of week 1 ($Z = -2.8$, $P < 0.01$). In week 2, the ant activity level at the treatment gel bait houses decreased to 16,918 ± 1,590 ant visits, while the standard gel bait house had a slight increase to 16,727 ± 1,644 ant visits, leaving the treatment and standard houses similar in their ant activity levels ($Z = -0.8$, $P = 0.4$). By week 4, the ant activity level of the treatment gel bait houses further decreased to 5,108 ± 753 ant visits, being significantly different from that of the standard gel bait houses (12,739 ± 1,253 ant visits; $Z = 4.7$, $P < 0.01$). When compared with their own pretreatment values, the treatment and standard gel baits achieved 74 and 42% overall reductions in the Argentine ant activity level, respectively.

Discussion

Data from the laboratory study clearly indicate that the addition of (Z)-9-hexadecenal into the gel bait increases Argentine ant foraging activity on the gel bait. This result is corroborated by the findings of Greenberg and Klotz (2000), in which consumption rate of Argentine ants was 33% higher when the sucrose solution was dispensed from vials, which were treated with (Z)-9-hexadecenal. Furthermore, higher foraging activity for the pheromone-treated gel bait translates to higher final mortality. Because (Z)-9-hexadecenal is not insecticidal (Choe et al. 2014), the increase in the final mortality found in the pheromone-treated gel bait is attributed to the increasing number of Argentine ant foragers that are discovering and consuming the gel bait within the fixed amount of time. The consumption data from the field study also indicate that Argentine ants consistently consume more gel bait when it is treated with (Z)-9-hexadecenal (Fig. 2); the total amounts of gel bait consumed were 238 and 95 g for the treatment and standard gel baits, respectively.

The field study suggests ant activity levels from the treatment and standard gel bait houses behave differently over time. Before the gel bait application, the houses assigned to either treatment or standard gel baits had similar levels of ant activity (Fig. 3). By the end of week 1 posttreatment, the houses with the treatment gel bait had higher ant activity compared with the houses with standard gel bait. This phenomenon was primarily caused by the fact that ant activity level at the houses with the treatment gel bait increased between the pretreatment and week 1 posttreatment monitoring trials, while the houses with the standard gel bait showed a reduction in ant activity level during this period. It is possible that (Z)-9-hexadecenal originating from the treatment gel bait stations might have initially increased the number of foraging ants around the house including areas where the monitoring tubes were placed. Argentine ants are known to be attracted toward the source of (Z)-9-hexadecenal via odor-mediated anemotaxis (Van Vorhis Key and Baker 1982a,b; Choe et al. 2012, 2014). Suckling et al. (2008) reported that the number of Argentine ants significantly increased when (Z)-9-hexadecenal was released from a point source within the field site.

The ant activity level in the treatment gel bait houses dropped substantially during the second week of baiting, resulting in similar levels of ant activity between the treatment and standard gel bait houses (Fig. 3). By the end of week 4, the treatment and standard gel baits achieved 74 and 42% reductions in the ant activity level, relative to their pretreatment values. Based on weekly monitoring of an untreated field site, ant activity levels within Riverside area did not experience any natural decline throughout the entire field study period (K.F.W., unpublished data). Additionally, the average temperature range throughout the study was 21–29.5°C, which is well within the temperature range which would allow normal foraging activity of Argentine ants (Abril et al. 2010). Throughout the 4-wk baiting period, the amount of gel bait consumption was consistently higher for the treatment gel bait compared with the standard gel bait (Fig. 2). Based on this information and the data from the laboratory study, the higher control efficacy achieved by the pheromone-treated gel bait by the end of week 4 could be attributed to the higher amount of thiamethoxam delivered to the Argentine ant populations compared with the standard gel bait. However, it is also possible that (Z)-9-hexadecenal incorporated into the gel bait might have influenced the spread of thiamethoxam throughout the population of Argentine ants. For example, the presence of (Z)-9-hexadecenal in the ingested gel bait might influence the frequency of the trophallaxis between the original forager and other recipient ants. This aspect warrants further study.

The 74% reduction achieved in the current field study using the "pheromone-assisted baiting technique" in only 4 wk is particularly remarkable when considering the efficacy levels from other previous baiting trials with commercial products targeting urban ant populations. For instance, one field study indicated that the same gel bait product used in the current study (0.01% thiamethoxam) provided an average of 46% reduction in ant activity around the houses after 30 d (Australian Pesticides and Veterinary Medicines Authority [APVMA] 2007). This level of control efficacy is similar with what the current study has observed for the standard gel bait by the end of week 4 (42%). Klotz et al. (2007) reported that houses treated with liquid borate bait only attained a 44% reduction in ant activity around the house after 4 wk.

For practical applications in pest management, achieving an initial attraction of Argentine ants to low concentrations of (Z)-9-hexadecenal incorporated into the gel bait is paramount. Even though Argentine ants are generally attracted to a source of (Z)-9-hexadecenal from a distance, it has been also shown that high concentrations of (Z)-9-hexadecenal applied in point sources are repellant or disruptive to the foraging ants (Greenberg and Klotz 2000, Suckling et al. 2010). Maintaining an optimal concentration range of (Z)-9-hexadecenal being released from the gel bait (or the gel bait station) is necessary to maximize the attraction and subsequent bait consumption by the foraging Argentine ants. Once the bait is discovered and consumed by a group of foragers, it is likely that the initial foragers will recruit more ants using their own communication system (Flanagan et al. 2013). The current study demonstrates that 0.1 µg of (Z)-9-hexadecenal per 1 g of gel bait is effective in maintaining attraction within the small laboratory colonies for 30 min without being repellent. The same rate of (Z)-9-hexadecenal was effective in attracting the foragers into the bait stations in the field settings. That is, we often observed that foraging Argentine ants readily discovered the pheromone-treated gel bait inside of the bait stations within 5–10 min after installing the bait stations.

The results of this study demonstrate the proof-of-concept for the pheromone-assisted baiting technique, suggesting its potential as an effective management tool for Argentine ants in urban settings. This pheromone-assisted baiting technique builds upon existing Argentine ant baiting technology by maintaining or improving important bait attributes such as delayed toxicity, transferability, and nonrepellency through the addition of (Z)-9-hexadecenal. Considering the current price of synthetic (Z)-9-hexadecenal (US$36.75 for 1 g, Bedoukian Research, Inc.), the pheromone-assisted baiting technique could be an economically viable modification for existing bait products. Other bait products containing different active ingredients (with different modes of action or transfer) need to be examined to determine their potential for the pheromone-assisted baiting approach. Also, an attribute of the bait station to consider in the current study is that the insecticidal gel bait is contained in a 15-ml plastic tube, thus eliminating the potential of insecticide run off into urban watersheds. The bait tube cap had a small circular hole (3 mm diameter) drilled in the middle of the cap to limit the entry of nontarget organisms and water while permitting Argentine ant access. Underground bait stations were protected from homeowners, children, and pets, and 100% of the bait stations were retrieved at the end of study. As federal and state laws become more stringent on residual insecticide spray use, the pheromone-assisted baiting technique may provide an effective improvement to current control strategies.

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