

/// ANNUAL ANT CONTROL ISSUE

ATTRACTION

FATAL

The use of an attractant pheromone can maximize the impact of pesticide sprays targeting nuisance ants.

By Dong-Hwan Choe and Kathleen Campbell



Argentine ants trailing on a wire. (Photo: Dong-Hwan Choe, UC-Riverside, Entomology)

Studies of pheromones and other behavior-modifying chemicals (also called semiochemicals or infochemicals) can benefit practical pest management by maximizing the control of the target species while reducing the quantity of insecticide applied in the environment. Synthetic sex pheromones and other attractant chemicals have been successfully implemented to achieve mass trapping or mating disruption of pest insects in various agricultural and natural environments. In particular, the “lure-and-kill” method uses attractant pheromones combined with insecticides at the lure source, attracting the target pests to the location where the insects will be subsequently exposed to lethal doses of the insecticides. With a highly effective synthetic pheromone, it is now possible to develop a similar tactic targeting invasive Argentine ants in urban settings.

ARGENTINE ANT ATTRACTANT PHEROMONE. Social insects such as ants, honeybees, and termites use a diverse array of pheromones for organization and coordination of all aspects of their colony development and maintenance (Vander Meer and Alonso 1998). In particular, the trail pheromones of ants are known to play critical roles in their foraging and nest relocation activities. The trail pheromone of the Argentine ant has been the focus

of numerous studies because of its significance in the species' mass recruitment behavior. Cavill et al. (1979) first isolated and characterized (Z)-9-hexadecenal from Argentine ants, and concluded that (Z)-9-hexadecenal might be a component of the trail pheromone complex of the species. Although the deposition of (Z)-9-hexadecenal on the trail by recruiting ants has been called into question (Choe et al. 2012), the chemical has been studied primarily as a trail pheromone component of the Argentine ant (Van Vorhis Key et al. 1981; Van Vorhis Key and Baker 1982a, 1982b, 1982c).

Several studies have explored the possibility of using synthetic (Z)-9-hexadecenal to develop practical management strategies for Argentine ants. For example, one study suggested that (Z)-9-hexadecenal might increase the consumption of sugar-based liquid baits by Argentine ant workers when it is mixed with the baits (Greenberg and Klotz 2000). Other studies have tested if the application of synthetic (Z)-9-hexadecenal disrupted trail formation and foraging activity of Argentine ant populations in the field (Suckling et al. 2008, 2010, Tanaka et al. 2009, Nishisue et al. 2010, Sunamura et al. 2011). However, these studies have only focused on the possible disruption of ants by applying a large amount of synthetic (Z)-9-hexadecenal in the environment.

LURE-AND-KILL LAB STUDY. We explored the use of (Z)-9-hexadecenal to attract Argentine workers to an area treated with

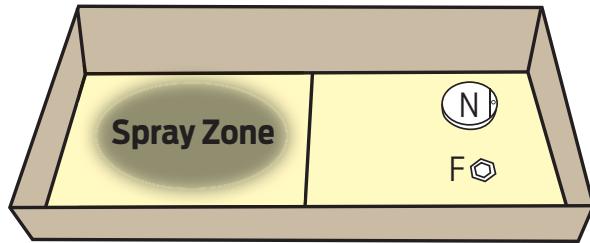


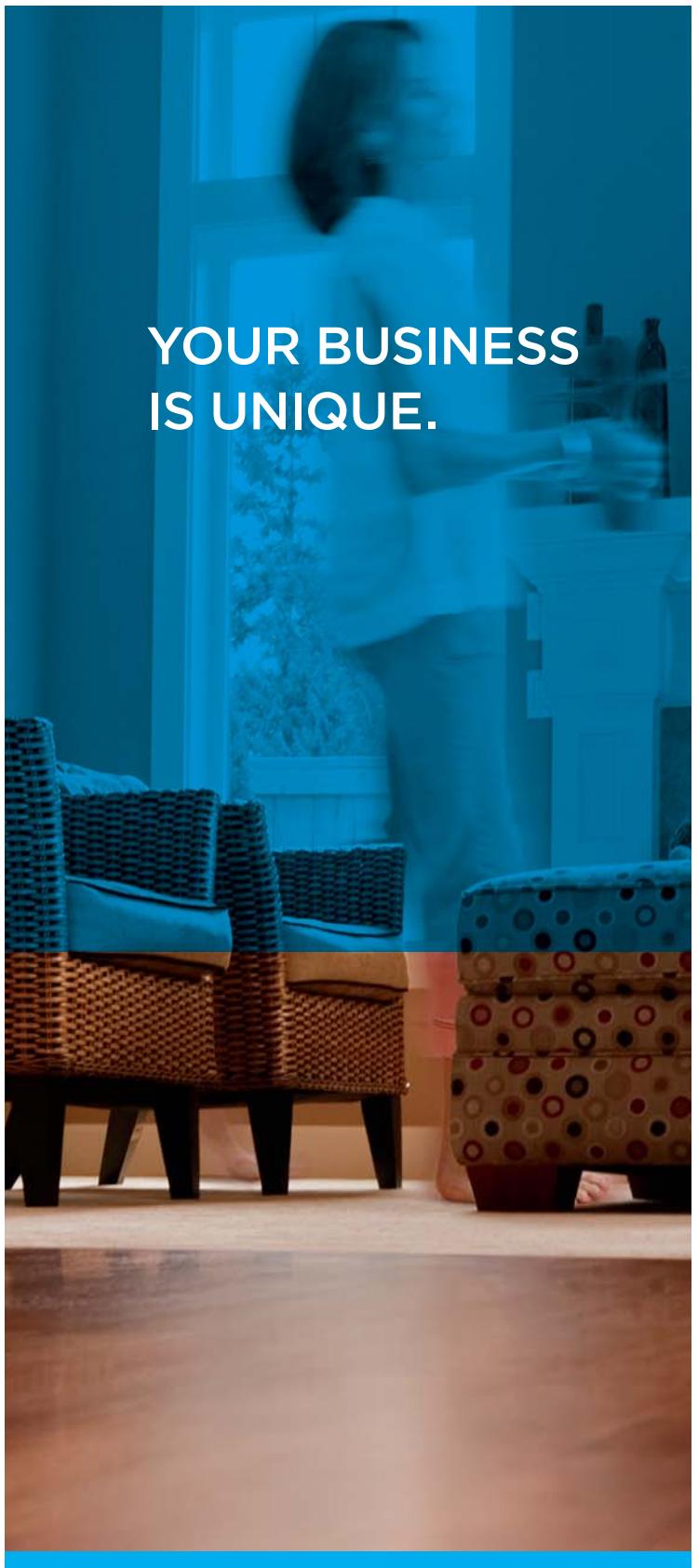
Fig. 1. Experimental setting for effect of pheromone on fipronil spray efficacy study. The shaded area on bottom of the box indicates the location where the insecticide spray was applied (spray zone). N and F denote nest and feeder, respectively.

insecticide, thereby maximizing the efficacy of insecticide sprays applied in the urban environment. This “lure-and-kill” approach utilizing the synthetic pheromone would be advantageous over conventional, stand-alone applications of insecticide spray because the insecticide/pheromone treated surface (soil, cement, wood) will attract foraging ants from nearby trails and even from the nest entrances and maximize the number of ants exposed to the treated surface.

First, to determine if the efficacy of an insecticide spray (fipronil) could be improved by adding the synthetic pheromone, (Z)-9-hexadecenal, laboratory studies were conducted. About 0.5 grams of Argentine ants from a laboratory stock colony was transferred into an experimental colony box (86 by 42 by 14 centimeters). The average number of ants in a colony was 731 ± 28.1 (mean \pm SEM, n = 6, range 600-782). All of the experimental colonies were provided at least one queen. Each colony box was provided with one artificial nest constructed from plaster-filled petri dishes (9 centimeters in diameter by 1.5 centimeters in depth), and a small plastic dish with 25% sugar water applied to a small piece of cotton. The nest and sugar water dish were placed on the same side of the box (Fig. 1). The entire bottom of the large colony box was covered with a thin layer of dry sand (400 grams), providing a more natural substrate for insecticide treatment.

Three different spray preparations were tested: (a) pheromone + fipronil, (b) fipronil only, and (c) pheromone only. 0.01% fipronil preparation was tested (1/6 of the label rate for ant control). For the spray combinations with pheromone [preparations (a) and (c)], we added 1 milligram of synthetic (Z)-9-hexadecenal dissolved in 1 milliliter of ethyl alcohol into 500 milliliters of deionized water. For the preparation (b), we added 1 milliliter of ethyl alcohol only. All of the spray preparations were mixed well in the spray bottle by shaking vigorously before application. A circular area of 491 cm^2 (25 centimeter diameter) in the opposite side of the nest and sugar water dish was treated using the hand sprayer, which delivers about 3 milliliters of liquid at a time (Fig. 1). The treated colonies were maintained at 70-77°F and 34-45% relative humidity. Mortality of the treated colonies was recorded daily for 7 days. The dead ants were removed from the colonies after being counted. Each treatment was replicated 5-6 times. Total cumulative number of dead ants

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was compared between treatments with a one-way ANOVA followed by Tukey HSD all-pairwise comparison test.

The application of fipronil and pheromone combination sprays resulted in significantly higher mortality in the laboratory colonies of Argentine ants than did the application of fipronil only. Day 7 mortality levels were significantly different between the three different treatments ($F = 48.8$; $df = 2, 13$; $P < 0.001$). The cumulative number of dead ants in the “pheromone + fipronil” treatment was significantly higher than that in the “fipronil only” treatment (458 ± 36.8 vs. 291 ± 27.1 , mean \pm SEM, $n = 5$ and 6, respectively) (Tukey HSD all-pairwise comparison test). The cumulative mortality of the “pheromone only” treatment at day 7 was 65.8 ± 11.0 (mean \pm SEM, $n = 5$), being significantly lower than the other two treatments with fipronil (Fig. 2, right).

FIELD STUDY. To determine if the lure-and-kill technique provide any improve-

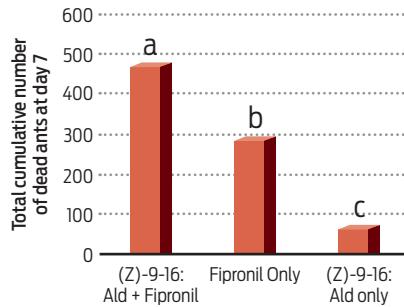


Fig. 2. Effect of (Z)-9-hexadecenal [(Z)-9-16: Ald] to the efficacy of fipronil spray. Comparison between pheromone + fipronil, fipronil only, and pheromone only are presented. The height of each bar indicates the mean cumulative mortality of ants (workers and reproductives) by 7. Means with different letters are significantly different at the $\alpha = 0.05$ level. See text for the details of statistical analysis.

ment in fipronil treatments targeting Argentine ant infestations under field conditions, a field study was conducted

with several volunteering houses in Riverside, California during summer 2013. Efficacy was calculated from a reduction in ant foraging, based on adjusted weight loss from monitoring vials of sugar water before and after treatment. Ten 15-milliliter polypropylene tubes filled with about 13 milliliters of 25% (weight/volume) sucrose water were placed around each structure for about 24 hours. After 24 hours, the tubes were sealed and returned to the laboratory and weighed. The homes were monitored 1 week before and 1, 2, 4 and 8 weeks after the treatment. Loss of liquid (i.e., weight) from the tubes was corrected for evaporation and drowned ants. The number of ant visits was calculated by dividing the consumption (grams) by 0.0003 grams/visit, a single Argentine ant consuming 0.0003 grams of sucrose water per visit (Reierson et al. 1998). There is a direct relationship between amount of sugar water consumed and the number of ant visits and the number of ants in the

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area. For example, lower numbers of visits represented lower overall foraging activity.

The sprays were applied using a 15-liter backpack sprayer; 0.06% fipronil was used for perimeter treatments (1 foot up, 1 foot out from structure foundation). The area where garage door contacts driveway was treated with pin-stream application (1-inch width). One gallon of preparation was made at a time; 0.75 milliliters of a ready-to-use formulation of (Z)-9-hexadecenal (16.9% wt, provided by Suterra) was mixed with the 1 gallon of preparation.

The number of ant visits from 10 monitoring stations were averaged per house per day, and the average values were used as representative data. The percentage of reduction of foraging activity was calculated per house per day per treatment based on the initial pre-treatment data. Because different houses had different levels of ant foraging activity, the post-treatment data were standardized for statistical analysis by converting them to the proportions of their own initial pre-treatment values. The proportion data on each day (i.e., 1, 2, 4, and 8 week post-treatment) were compared between treatments (i.e., insecticide only vs. insecticide + pheromone) with nonparametric Wilcoxon Rank Sum Test with $\alpha = 0.05$.

The 0.06% fipronil only treatment initially had modest to good control by providing 48-86% reduction by week 2 (Table 1, page 56). However, fipronil only treatment failed to provide any reduction in one of five houses at weeks 4 and 8 (see House 4 in Table 1). By the end of week 8, the fipronil only treatment had widely variable efficacies, ranging 0-65% reduction, with 17% reduction on average (Table 1). In contrast, fipronil + pheromone treatment initially had good to excellent control by providing 84-98% reduction by week 2, and maintaining 42% reduction on average at week 8 (Table 2, page 56). All houses with fipronil + pheromone treatment had $> 32\%$ reduction throughout the 8-week study (Table 2). There was significant difference in ant foraging activities between fipronil only and fipronil + pheromone treatments at weeks 1 and 2 (Fig. 3).

IMPROVED EFFICACY. Our study showed that the presence of an attractant pheromone incorporated in an insecticide spray can attract Argentine ants from nearby locations to the insecticide spray deposits. Our lab studies with fipronil sprays demonstrated the addition of (Z)-9-hexadecenal in the insecticide sprays increased the insecticidal efficacy by 57% based on the cumulative mortality of ants. Because the pheromone was not insecticidal by itself (Fig. 2, page 51), the improvement in efficacy could be attributed to the attraction of ants from



Argentine ant workers on sand. Equipped with extremely sensitive sensory organs (e.g., antennae), these worker ants are capable of detecting very low concentrations of their pheromones in the air. (Photo: Dong-Hwan Choe, UC-Riverside, Entomology)

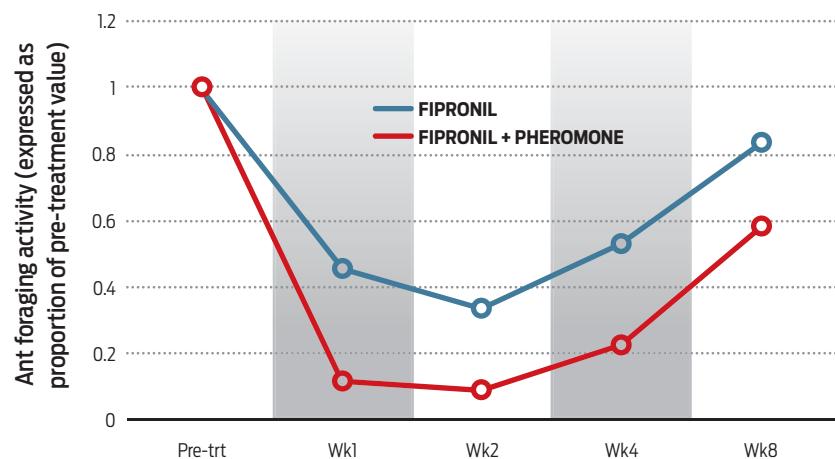


Figure 3. Comparison between Fipronil only and Fipronil + Pheromone sprays. Post-treatment activity levels are expressed as proportions of corresponding pre-treatment activity levels. Data are averaged from five houses per treatment. For weeks 1 and 2, there is significant difference in ant foraging activity between Fipronil only and Fipronil + Pheromone treatments (Wilcoxon Rank Sum Test: $\alpha = 0.05$).

nearby locations, and subsequent exposure of the ants to lethal doses of the insecticides. We propose three possible advantages of this lure-and-kill tactic. First, the insecticide spray deposits not directly applied on the active trail or nesting sites will still provide good control by attracting the ants to the insecticide deposits. Second, the insecticide and pheromone directly applied on

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Table 1. Pre- and post-treatment ant visits in fipronil only treatment.

HOUSE	PRE-TREATMENT AVG. ANT VISITS	POST-TREATMENT AVG. ANT VISITS (% REDUCTION)			
		1WK	2WK	4WK	8WK
HOUSE 1	36,789	14,280 (61.2%)	19,127 (47.8%)	22,107 (39.9%)	34,564 (6%)
HOUSE 2	30,826	13,535 (56.1%)	4,462 (85.5%)	6,546 (80.8%)	26,089 (15.4%)
HOUSE 3	31,726	14,752 (53.5%)	11,042 (65.2%)	13,222 (58.3%)	11,254 (64.5%)
HOUSE 4	28,564	18,499 (35.2%)	12,459 (59.7%)	34,630 (0%)	37,582 (0%)
HOUSE 5	32,211	11,128 (65.5%)	6,920 (78.5%)	7,223 (77.6%)	23,224 (27.9%)
AVERAGE	32,023	14,439 (54.9%)	10,802 (66.3%)	16,746 (47.7%)	26,543 (17.1%)

active trails and the nest entrance might maximize the transfer of the insecticide to the target pests. Preliminary observations from the lab and field indicate Argentine ant workers attracted to the pheromone deposit stayed within the treated area for an extended period of time, e.g., 10 minutes (D.-H. Choe, unpublished data). Increasing the time that ants contact the treated

surface will allow us to reduce the quantity of insecticide applied in the environment without losing its efficacy to control the target pestants. Third, the pheromone-assisted technique will increase the likelihood of ants contacting the insecticide residue before any significant degradation of the active ingredient of insecticide occurs on the treated surface.

FUTURE APPLICATIONS. From a practical standpoint, a future development of proper formulation of the (Z)-9-hexadecenal would improve its efficacy and usability. Field conditions, such as direct sunlight and extreme temperature could negatively influence the persistence of the pheromone. The physicochemical characteristics of the pheromone for-



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Table 2. Pre- and post-treatment ant visits in fipronil plus pheromone treatment.

HOUSE	PRE-TREATMENT AVG. ANT VISITS	POST-TREATMENT AVG. ANT VISITS (% REDUCTION)			
		1WK	2WK	4WK	8WK
HOUSE 1	29,457	3,051 (89.6%)	611 (97.9%)	7,493 (74.6%)	16,760 (43.1%)
HOUSE 2	37,809	4,536 (87.9%)	4,296 (88.6%)	5,104 (86.5%)	25,481 (32.6%)
HOUSE 3	39,444	9,076 (77%)	6,255 (84.1%)	15,479 (60.8%)	26,195 (33.6%)
HOUSE 4	43,388	3,263 (92.5%)	1,777 (95.9%)	5,420 (87.5%)	22,142 (49%)
HOUSE 5	43,414	2,327 (94.6%)	5,635 (87%)	9,479 (78.2%)	22,160 (49%)
AVERAGE	38,702	4,451 (88.5%)	3,715 (90.4%)	8,595 (77.8%)	22,548 (41.7%)

mulation would be important factors in improving the persistence of the effect. Proper packaging may be necessary because the stability of the pheromone might be negatively affected if it is mixed with the insecticide formulation for long-term storage. The pheromone and insecticide formulations might be prepared in the required concentrations in a solvent, packaged in separate containers, and mixed prior to use in the field. **PCT**

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