

**Low-Toxic Control of Argentine Ants Using Pheromone-Enhanced Liquid Baits**

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## **Introduction**

Good pest management is based on a thorough understanding of a pest's behavior and how that knowledge can be used to control them. First, and foremost in ant control we have been steering toward reduced pesticide use, by developing baits to use in place of sprays, which exploit the ant's natural foraging behavior instead of relying on contact insecticides which kill indiscriminately the pests as well as beneficial organisms. We have developed delivery systems for baits in place of broadcasting them, capitalizing on the ants' recruitment behavior. We are using very low doses of toxicants in our baits so that they are slow-acting, ensuring their distribution throughout the colony.

To attain these goals of good pest management our research has focused on the control of Argentine ants. Argentine ants were first reported in California in the late 1800's and since that time have become the number one urban pest in southern California. Currently, pest control operators rely heavily on perimeter treatments with contact insecticides to control them.

Our research has been exploring outdoor use of liquid and granular baits for ant control in the structural environment.

In our first field test described in Chapter 1, we used 0.5% boric acid in 25% sugar water as bait to control a heavy infestation of Argentine ants at a water production facility in Huntington Beach. The delivery system we developed for this bait consisted of clear plastic tubes with a latticework inner matrix for the ants to crawl and feed on. This bait station was placed inside a slightly larger PVC pipe to protect it. Bait stations were placed around the outside perimeter of buildings. We noticed immediately that complaints about ants inside the buildings ceased. All of the ant activity was now directed to the bait stations outside. We conducted our bait test for 10 weeks and eventually achieved over 80% reduction in ant activity in the treated versus the control sites. This measurement of ant activity was based on counts of ants entering the bait stations over a one minute time interval.

In our next field test in Huntington Beach (Chapter 2) we evaluated two granular hydramethylnon baits against Argentine ants. Our test sites were fire stations and water substations. The first bait we used was Amdro which contains soybean oil on corngrit with 0.73% hydramethylnon as the active ingredient. After two weeks we made a second application with a protein-carbohydrate blend bait with 0.9% hydramethylnon. We compared a broadcast application of the bait to bait delivered in stations. We measured consumption of sugar water to

estimate ant activity. We allowed the ants to feed on our sugar water monitors for over 24 hours and then calculated milliliters consumed per hour. This gave us a more accurate estimate of ant activity than counting ants for one minute at a particular time of the day.

The Amdro with soybean oil applied after the pretreatment measurement had no significant effect on ant activity as measured by sugar water consumption. The protein-carbohydrate blend bait applied after 2 and 5 weeks, however, significantly reduced activity as measured by sugar water consumption. The ant activity in the control sites stayed relatively high. This study showed that baits in stations are just as effective as broadcasted baits. Another important insight from this study is that soybean oil on corngrit is ineffective against Argentine ants but highly effective against the red imported fire ant. There is increasing anecdotal evidence that Argentine ants are slowing the spread of imported fire ants in certain areas of California. By using selective baits under certain conditions Argentine ants may achieve a competitive edge thereby tipping the scale against imported fire ants.

In our last field study, Chapter 3, we combined a granular and liquid bait to provide a more complete diet for the Argentine ants. We used Maxforce Fine Granules as a protein source and sugar water as a carbohydrate source to compare with a spray treatment for Argentine ants around houses in the Riverside area. Bait stations with Maxforce granules were placed alongside our liquid bait stations containing a half percent TIM-BOR in 25% sugar water. Our estimate of ant activity was even more precise than the volumetric method used previously. This time we used a gravimetric technique. Vials of sugar water were weighed and then placed around the outside perimeter of houses. Twenty four hours later they were collected and weighed again to determine how much was consumed. Based on the amount of sugar water that one ant can consume it is possible to calculate the number of ant visits to one of these monitoring vials over a 24 hour period.

This segment of our research demonstrated the long-term effectiveness of a thorough spray treatment. One application of a contact insecticide gave at least 10 weeks control. The baits were ultimately as effective, but it took longer to get control and they had to be maintained on a weekly basis, a heavy investment of time and energy. This type of baiting program would be cost-prohibitive for a pest control business and underlines the fact that we still have a long ways to go before a baiting strategy for Argentine ants can compete in the marketplace with sprays.

In Chapter 4 we describe laboratory and field studies to investigate toxicity and repellency of borate compounds in 25% sugar water. These studies provide us with information on how to formulate liquid baits that use borates as the toxicant. We found that the toxicity of TIM-BOR is similar to other boron compounds such as boric acid and borax. Each of these compounds have different amounts of boron. However, when lethal times of Argentine ants are graphed against boron concentration of TIM-BOR, boric acid and borax they all fall along the same curve. TIM-BOR, however, is much easier to dissolve in water than boric acid or borax making formulation much easier with this compound.

Two of the most promising areas of research lie in the realm of pheromones and habitat modification. In Chapter 5 and 6 we describe two studies with Argentine ants where we were able to increase consumption of a bait: 1) by the addition of their trail pheromone; and 2) by creating a physical barrier to the ants.

Both of these areas of research are new techniques in ant control. We know that ants contain a battery of exocrine glands for various kinds of pheromones, and that olfaction is a primary sensory system of ants. This provides an opportunity to develop more attractive baits. Pheromone enhancement could be used to develop baits that are target-specific and therefore environmentally safe.

In habitat modification we create inhospitable or less than optimal living conditions for ants, e.g. by control of homopterans which ants are using as a major food source. We've conducted experiments on the use of sticky barriers on trees to cutoff Argentine ants from their honeydew source. Baits placed beneath these barriers have increased consumption compared to baits in trees without barriers. Dr. Phil Phillips and the late Dr. Harry Shorey used strings soaked in repellents like farnesol as barriers to Argentine ants in citrus trees. The big challenge to the pest control industry is designing these approaches to make them practical and cost-effective.

No other household pest challenges the pest control industry to the extent that ants do. To control them pest control operators need to understand their cryptic nature, fastidious feeding habits, and dynamic colony structure. Research is the key to gain an understanding of their behavior and education the means to controlling them.

## **Chapter 1: Liquid Boric Acid Bait for Control of the Argentine Ant**

Argentine ants, *Linepithema humile* (Mayr), are a unicolonial, polygynous "tramp" species specialized to support large populations that extend over an entire habitat (Hölldobler and Wilson 1990). In an urban setting their nests are spread over a wide area and are considered one colony with numerous nests (Passera 1994). Their colonies have a large capacity for growth, due to numerous queens and colony fission (Majer 1994). These characteristics, along with their propensity to live in close association with humans, have contributed to their ecological success (Passera 1994). In California, Argentine ants are the most common urban ant that pest control operators are asked to control in and around structures (Knight and Rust 1990).

Control of Argentine ants includes the application of baits (Rust and Knight 1990) and residual chemical barriers (Rust et al. 1996). Unfortunately, baits have shown only marginal efficacy (Hedges 1997). Barrier treatments kill many of the beneficial insects and may result in secondary pest outbreaks (Smith et al. 1996). In addition, because of the repellency of pyrethroids to Argentine ants (Rust and Knight 1990, Knight and Rust 1990), their use as barriers may disperse ants within structures and magnify control problems (Rust et al. 1996).

A major problem in developing baits for Argentine ants has been formulating toxicants into liquid sucrose baits that are highly preferred by this ant (Baker et al. 1985). A 5% boric acid bait has been tested against Argentine ants but it was not readily accepted, and killed less than 50% of the workers (Rust and Knight 1990). Previous laboratory studies demonstrated the potential of low concentrations (1%) of boric acid in sucrose water for control of Argentine ants (Klotz et al. 1996). Our objective in this investigation was to further refine a boric acid--sucrose water bait to be used against Argentine ants by (1) finding the optimal concentration of sucrose in water to attract ants, (2) determining the dose response of ants to different concentrations of sucrose and boric acid solutions, and (3) based on the information obtained from (1) and (2), selecting a formulation of the boric acid--sucrose water bait to evaluate in the field against a structural infestation of ants.

### **Materials and Methods**

**Recruitment Tests.** Granulated sugar was dissolved in deionized water to produce solutions of

10, 25, and 50% (wt:vol) sucrose water. A droplet of each solution (100  $\mu$ l) was added to a microscope slide depression. Slides with 2 different concentrations of sucrose water were placed side by side inside a 44 by 56 cm plastic nesting tray (Panel Controls, Detroit, MI) .30 cm from a plastic rearing cell (100 diam. by 15 mm petri dish). The rearing cell contained at least 1 queen and several hundred workers that were collected on the previous day from a citrus grove on the UC Riverside campus (Riverside Co., CA). To prevent ants from escaping, the inside walls of the nesting trays were coated with Fluon (ICI Americas, Inc., Bayonne, NJ). The ants were provided water but no food for 1 d prior to bait exposure. Recruitment to the sugar solutions on the 2 slides was determined by counting the number of ants at each of the 2 slides at 3-min intervals for 1 h. For each pair of sugar concentrations tested (10 versus 25 and 25 versus 50%), 3 different colonies were replicated simultaneously.

**Toxicity Tests.** Argentine ants were collected as above. In the laboratory the ants were provided water but no food. One day after collection, workers were distributed, 10 each, into small Gelman petri dishes (50 diam. by 9 mm, Fisher Scientific, Ann Arbor, MI), each supplied with a cup-shaped plastic lid cut from a microcentrifuge tube (1.5 ml, Fisher Scientific, Pittsburgh, PA). The concave side of the plastic lid was plugged with cotton and plastic adhesive (Holdit™, Faber-Castell Corp., Lewisburg, TN) was used to anchor the flat side of the lid to the center of the bottom of a petri dish. A 1.6-mm hole was drilled in the center of the petri dish lid so that bait solutions could be added with a micropipette to the cotton plug without having to remove the lid and disturbing the ants. Crystalline boric acid (99% [AI], Sigma, St. Louis, MO) was dissolved in 10 and 25% (wt:vol) sucrose-deionized water solutions to produce 5 concentrations (0.2--1%) of boric acid for each of the 2 sucrose solutions. Treatments and controls (10 and 25% sucrose-deionized water) were replicated 5 times. The bait solutions were available continuously to the ants for the duration of the test. Daily observations on cumulative mortality were recorded for 9 d.

**Field Tests.** Field trials were conducted to determine the efficacy of a boric acid--sucrose water bait against an infestation of Argentine ants at a water production plant (2.8 ha) in Huntington Beach (Orange Co., CA). The plant has 3 single-story buildings and 2 large reservoirs (Fig. 1). An initial survey (before treatment) for Argentine ants was conducted on the outside perimeter of

the plant's boundary with Huntington St. and Garfield Ave., around buildings and along 1 side of a reservoir (Fig. 1). Small Gelman petri dishes were baited with honey (.1 g) and placed on the ground, at 12 m intervals along the edge of vertical wall surfaces. Counts of the number of ants feeding on the honey were made .2 h later. Based on this pretreatment survey and information provided by the plant operation's manager, bait stations were placed around the perimeter of the buildings up against the foundation, and along 1 side of a reservoir. Nineteen bait stations for the treatment area were filled with 0.5% boric acid in 25% sucrose water and assigned to 2 of the buildings with the heaviest infestation. Sixteen control stations (25% sucrose water) were assigned to the other building and alongside the reservoir where the infestations were lighter. Bait stations were constructed from a 23 cm length of 4.2-cm-diameter transparent acrylic tube, filled with a strip of evaporative cooler pad (Coolpad, Research Products, Phoenix, AZ) as a matrix to allow ants to feed, and sealed at both ends with plastic caps. Three 1.1-cm holes were drilled along the length of the tube to permit ants access to the bait. The station was placed inside a 25 cm length of 6-cm-diameter polyvinyl chloride pipe as a sleeve to hold the bait station, to provide shade during hot weather, and to protect it from rain and watering. An 8 mm gap between the upper surface of the bait station and the sleeve prevented non-target animals from gaining entry to the bait. Bait stations were refilled once per week for 10 wk.

Ant populations were monitored weekly for 10 wk by counting the number of ants entering the bait stations over a 1-min interval. A final survey was conducted at the end of the study to compare populations in the treatment and control areas. At that time all of the bait stations were replaced with clean, fresh stations filled with 25% sucrose water and no boric acid. On the following day counts were made of the number of ants entering the bait stations over a 1-min interval.

**Statistical Analysis.** Recruitment of ants over time to different sucrose solutions was analyzed using a paired *t*-test comparison (StatView 1992). For toxicity tests, mortality data were corrected with Abbott's (1925) formula and analyzed by probit analysis (Raymond 1985) to determine lethal times (LT50 and LT90) for each concentration.

The Huntington Beach data are highly skewed to the right due to very high counts in a few of the bait stations. Log transformation of these data greatly reduces skewness and also improves the spread of residuals and variances, allowing the use of parametric tests in the

analysis (Systat 1997). For each bait station we calculated the ln (natural log) transform of the number of ants entering the trap + 1. Using all these transformed numbers we performed linear regressions of the number of ants entering the traps as a function of time (Fig. 2 only shows means). We also used the transformed data in a repeated measures analysis of variance (Systat 1997). The latter gives an interaction term for time\*treatment.

## Results and Discussion

Markin (1970) estimated that 99% of the food entering Argentine ant colonies consists of honeydew and nectar. Sucrose usually predominates in honeydew (Tennant and Porter 1991) and is an important component of nectar (Baker and Baker 1975, Gottsberger et al. 1984). In laboratory tests Baker et al. (1985) found that Argentine ants preferred 25% sucrose or honey water over other solid foods such as granulated brown sugar or those with high protein content. Laboratory tests demonstrated the efficacy of a 10% sucrose water bait against Argentine ants (Klotz et al. 1996), but based on information from M. K. Rust (UC Riverside, personal communication), baits with higher concentrations of sucrose are even more attractive. Recruitment tests showed a preference of the ants for 50 > 25 > 10% sucrose in water. The number of ants (mean  $\pm$  SEM) feeding on 50% sucrose water was  $20.1 \pm 1.6$ , and on 25%,  $16.3 \pm 1.3$ . There was significantly more recruitment to the higher concentration of sucrose (paired  $t$ -test = -5.9,  $df = 19$ ,  $P < 0.0001$ ). In a test between 25 and 10% sucrose in water, the number of ants recruited were  $42.6 \pm 2.7$  and  $32.1 \pm 2.0$ , respectively, with significantly more ants feeding on the higher concentration of sucrose (paired  $t$ -test = -9.0,  $df = 19$ ,  $P < 0.0001$ ). Based on field trials by H. H. Shorey (UC Riverside, personal communication) in citrus groves, high concentrations of sucrose in baits (>25%) resulted in crystallization of the sugar, which interfered with bait delivery to Argentine ants. Therefore, we formulated a bait for field testing with 25 rather than 50% sucrose.

A slow-acting toxicant is a prerequisite for an effective ant bait (Stringer et al. 1964). The dose response of Argentine ants to low concentrations of boric acid (?1%) exhibits delayed toxicity (Table 1). At progressively lower doses, a slower mortality or higher LT50 and LT90 were obtained. The lethal times for Argentine ants were lower than those for Florida carpenter ants, *Camponotus floridanus* (Buckley), at similar concentrations of boric acid (Klotz and Moss 1996). This difference in dose response probably is due to the much larger size of carpenter ants

compared with Argentine ants. The confidence limits (CLs) for lethal times (LTs) of the 2 sugar solutions (10 and 50%) at a given concentration of boric acid overlapped in all cases except for the LT90s at 0.8 and 1%, where the speed-of-kill was quicker for the ants exposed to boric acid in 10% sugar water (Table 1). A similar trend exists for several of the other concentrations of boric acid although the confidence limits overlap. This difference in LTs may simply result from a reduced consumption of the bait with more sugar.

Recent laboratory tests with Argentine ants showed that when colonies were exposed continuously to 0.25, 0.5, and 1% boric acid in 20% sucrose water, only the 0.5% provided 100% mortality of workers and queens (M. K. Rust, UC Riverside, personal communication). And because our toxicity tests showed minimal to no difference in lethal times of ants exposed to the same concentration of boric acid in 10 or 25% sucrose water, we formulated a 0.5% boric acid bait for field testing.

Figure 2 shows the regression analysis of the Huntington Beach field experiment. The regression coefficient for the controls without boric acid is not significant ( $n = 154$ ;  $df = 1,152$ ;  $F = 0.53$ ;  $P = 0.47$ ) over the course of the experiment. In contrast, the regression coefficient for the experimentals with boric acid is highly significant ( $n = 182$ ;  $df = 1,180$ ;  $F = 63.9$ ;  $P \ll 0.001$ ;  $r^2 = 0.26$ ; slope  $\pm$  SE =  $-0.308 \pm 0.017$ ; intercept  $\pm$  SE =  $4.6 \pm 0.1$ ). In the repeated measures anova, the time by treatment interaction was also significant ( $P < 0.001$ ). This implies that the slopes of the 2 regression lines are not the same.

In the field test, an immediate result of the bait application was a reduction of complaints by employees working inside the buildings. Ant foraging activity had been redirected from the inside of buildings to the outside, an effect also noted by Forschler (1994b) in his field tests with containerized bait stations placed around the outside of buildings. Ant populations around the control stations (25% sucrose water) did not show a significant reduction over the duration of the test, whereas a steady and continuous reduction of ants occurred around the buildings treated with boric acid. The mean number of ants per trap during wk 1--3 was  $95 \pm 11.2$ , whereas for wk 8--10 it was  $18 \pm 3.9$ , a reduction of 81%. In the control areas for the same time periods the respective counts were  $51 \pm 15.0$  and  $34 \pm 7.1$ , respectively, or a reduction of 33%. The final survey with fresh, nontoxic bait stations gave mean counts of  $37 \pm 9.0$  ants for the boric acid treatment area and  $32 \pm 7.9$  for the control area. This boric acid treatment area count is still much lower than the 3-wk beginning average given above.

Due to the large initial ant populations and the continuous arrival of new colonies replacing those killed by toxic bait, a complete elimination of ants was not attained. Rather, the Argentine ant problem was managed, in that ants were restricted to foraging outside while their numbers were steadily reduced. Ideally, a management program of this kind would begin in the spring before ant populations reach the high density that typically occurs later in the summer and fall.

The low concentration of boric acid in a liquid form coupled with the high density of ants in this infestation required that large volumes of bait be continuously available to the ants. The number of ants entering stations during 1-min intervals could be as high as 315. Each bait station held 230 ml of bait, but after 1 wk there were always several empty stations. Therefore, bait formulation and the amount of bait available to the ants were key factors to the success of this management program.

## **Chapter 2: Evaluation of Two Granular Baits for Control of Argentine Ant**

For household ant control, most commercial baits are contained in child-resistant bait stations designed for indoor use (Hedges 1997). Recent studies with Argentine ants, *Linepithema humile*, have demonstrated the effectiveness of outdoor applications of containerized baits (Blachly and Forschler 1996; Forschler and Evans 1994a,b). Since most ant infestations originate from outside buildings (Hedges 1997) outdoor applications may be an effective way to prevent the entry of ants into structures. In addition, outdoor applications are less disruptive to the homeowner, because pest control operators can limit their activities to the outside of a residence (Oi et. al 1994).

In a previous study to control structural infestations of Argentine ants (Klotz et. al 1998), the authors made outdoor applications of an experimental liquid bait consisting of 0.5% boric acid in 25% sucrose water delivered in high capacity (230 ml) bait stations. Results of this study showed a gradual reduction in the number of ants in the treated versus the control areas over the 10 wk duration of the test. Even though slow-acting, the baits still had an immediate effect, because the ants causing problems inside the buildings were attracted outside to feed on the highly concentrated resource (25% sugar water). The reduction of ant activity inside due to the shift in foraging to the outside was also noted by Forschler (1997) in his work using

containerized solid baits to control Argentine ants around an apartment complex.

In our search for faster-acting outdoor baits for Argentine ant control, we report here on field tests of two kinds of granular bait with hydramethylnon as the active ingredient. We also investigated bait delivery in order to compare the efficacy of a point source application using bait stations to a broadcast application of the granules.

## **Materials and Methods**

**Baits.** Experimental baits consisted of 0.73% hydramethylnon formulated in soybean oil on a corn grit base (Amdro Insecticide Bait) and 0.9% hydramethylnon in silkworm pupae (Amdro Lawn and Garden Ant Bait) both provided by American Cyanamid (Princeton, New Jersey).

**Preliminary Field Test.** The site for a pilot test was located in residential housing in Riverside (Riverside County, CA). A survey was conducted first to find trees around homes with foraging trails of Argentine ants. From this initial survey, eight trees were chosen for the test. Argentine ant activity was estimated by counting for three minutes the number of ants travelling up a tree on an established trail.

To evaluate their efficacy in reducing ant activity, the two baits were randomly assigned to eight trees so that four trees were used per treatment. Pretreatment activity was determined from the survey reported above. Then, five tablespoons of bait were scattered at the base of each tree directly under the trailing column of ants. Post-treatment activity was monitored 3, 7 and 14 days after treatment, following the same procedure as the pretreatment counts. Extended post-treatment intervals were not surveyed due to migration of new Argentine ant colonies into the treatment sites after two weeks.

**Comprehensive Field Test.** After the pilot test, a comprehensive test was designed to investigate efficacy of baits against structural infestations of Argentine ants. Test sites consisted of three fire department stations and four water production substations located in Huntington Beach (Orange County, CA). Two of the fire stations were of sufficient area to accommodate two treatment sites, so that the test included nine sites in all, which received three different treatments. Three sites received: (1) a broadcast application of bait; (2) bait delivered in stations; and (3) no bait, and served as controls. Estimates of ant activity for each site were

based on consumption of 25% sugar water inside plastic centrifuge tubes (50 ml) fitted with Weed Block, a perforated plastic material which allowed the ants to drink. To make these monitoring tubes a 13/16" diameter hole was drilled through a 1" diameter centrifuge cap. A 1-1/4" square of weed block was centered over the top of a centrifuge tube filled with sugar water and then the cap was screwed down over the weed block to secure it in place. The monitoring tubes were inverted and taped at eye level to building walls at approximately 20 ft. intervals around the outside perimeter. At sites with bait stations, the monitoring tubes were placed on the wall at the same locations as the bait stations on the ground below which were removed during monitoring. Using a spray bottle, 25% sugar water was sprayed from the bottom of the tube down to the base of the building wall to give ants on the ground a path up to the tube. After approximately two days, the amount of sugar water consumed by the ants in each of the vials was recorded. The total volume consumed per tube was divided by the number of hours the ants were allowed to feed, to determine the ml of sugar water consumed per hour. A pretreatment survey was conducted to assess population density at each of the nine sites. After the pretreatment survey, bait applications were made at the label rate for Maxforce, namely 1 oz. per 50 linear feet, measured around the outside perimeter of the buildings to be treated. In the broadcast treatment, baits were spread along a 1 ft. wide band around the building. Bait stations consisted of B&G Perimeter Patrol Systems (B&G Equipment Co., Plumsteadville, PA) either duct taped on horizontal concrete surfaces or placed on the ground, spaced at approximately 20 ft. intervals around the building. Each bait station at a site contained the same amount of bait, determined by  $[(\text{total linear feet of the building perimeter}/50) \times 1 \text{ oz.}]/\text{no. of bait stations}$ . At control sites consumption of sugar water was monitored by the same method used at sites which received bait. This monitoring procedure was conducted on a weekly basis for 8 weeks. Mean hourly rates of sugar water consumption were calculated each week for each of the three treatments. Baits were applied on three different dates: the first application with Amdro Insecticide Bait was made the day after the pretreatment survey; the second and third applications with Amdro Lawn and Garden Bait were made between week 2 and 3 and 5 and 6.

**Statistical Analysis.** For the preliminary field test a Repeated Measure design (Systat 1997) was used on a square root transformation of the number of ants counted on trails. The transformation improved the equality of variances among the groups, required by Analysis of

Variance. For the comprehensive field test, a mean consumption of sugar water (ml/hr) for the control and treated buildings was evaluated by Analysis of Variance (Systat 1997) for 3 sample dates: (1) before application of baits; (2) 2 weeks after the first bait application; and (3) at the end of the test. Means were separated using Tukey's highly significant difference (HSD).

## **Results and Discussion**

In the preliminary field test both Amdro Insecticide Bait and Amdro Lawn and Garden Bait significantly reduced ant activity ( $P < 0.001$ ). The lack of significance in the interaction term, time\*treatment, meant that the rates of reduction in ant activity for the two baits were not significantly different from each other (Fig. 3).

The application of the Amdro Insecticide bait in the comprehensive field test had no significant effect on ant activity after 2 weeks when compared with the control ( $F = 2.402$ ,  $df = 2,43$ ,  $P = 0.1026$ ). At 6 weeks, after two applications of Amdro Lawn and Garden Bait, ant activity was significantly reduced in both the broadcast and bait station sites when compared with the controls ( $F = 12.1136$ ,  $df = 2,41$ ,  $P = .00007$ ). The bait station treatments were not significantly different from the broadcast application ( $P = 0.99$ ) but both treatments were significantly different from the control ( $P = 0.0005$  and  $0.0001$ , respectively).

Old bait stations were replaced with new ones on each of the 3 different bait applications. Large amounts of bait remained in all of the old stations, indicating that label rates of application were too high. Stations have several advantages over broadcast delivery of baits: (1) exploitation of the Argentine ants foraging behavior whereby ants use trail pheromones to recruit nestmates to a clumped versus scattered resource (Van Vorhis Key); (2) small amounts of toxicant can be placed in discrete locations, minimizing potential exposure to humans and pets (Knight and Rust 1991); and (3) protection of the bait from rain or sprinklers and direct sunlight. The latter is particularly significant because hydramethylnon degrades within days when exposed to sunlight (Vander Meer et al. 1982).

Two weeks after application of Amdro Garden Ant Bait only the sites with bait stations had significantly less ant activity than the control. In an attempt to eradicate the ants, a second application of Amdro Garden Ant Bait was made after the fifth week of the test. However, ant activity, albeit minimal, continued for the duration of the test.

The gradual reduction of ant activity at all sites during the first 3 weeks of the test, and

the upsurge in activity in the control and broadcast sites afterwards, were probably caused by climatic factors such as temperature and rainfall. This period was unusually cool and rainy for August.

The difference in bait efficacy was probably due to differences in their attractancy. Our observations of the ants' behavior during bait application supports the hypothesis that their attraction to and collection of Amdro Insecticide Bait was minimal, while for the Amdro Lawn and Garden Bait, the ants were highly attracted to it and readily collected it. In mound treatments with Amdro Insecticide Bait, Wagner (1983) reported that Argentine ants did not accept it and colonies were unaffected by it. For species of ants where acceptance was excellent, for example *Pogonomyrmex californicus* and *Veromessor pergandei*, ant activity in nests ceased within 48 hours. The reduction in ant activity with Amdro Insecticide Bait in the preliminary test reflects a low level of bait acceptance, and a small population of ants at this test site compared to massive ant populations at the comprehensive test site. The reduced efficacy of the soybean oil bait may have important implications for the emerging problem in California with red imported fire ants, *Solenopsis invicta*. The Argentine ants' high population density in conjunction with minimal efficacy of fire ant baits using soybean oil may give the Argentine ant an advantage in interspecific competition with imported fire ants, thereby slowing down the latter's spread.

Preference by Argentine ants for protein (Maxforce) over soybean oil (Amdro) bait was also found by Krushelnycky and Reimer (1998b) in Hawaii. In their attempt to control Argentine ants in Haleakala National Park using Maxforce they reduced foraging ants by 97% two days after treatment (Krushelnycky and Reimer 1998a). They speculated that the main obstacles to their eradication efforts were the short exposure time to the bait and toxicant due to bait spoilage from molds, toxicant degradation by ultraviolet light and quick mortality associated with hydramethylnon. Knight and Rust (1991) also reported a quick kill with Maxforce. In their laboratory tests with Argentine ants, they reported 36% reduction in 24 hours, too fast to be considered a delayed action toxicant as defined by Stringer et al. (1964).

Based on our results and those from previous studies, protein-based baits appear to be good candidates for use against Argentine ants. Both Rust et al. () and Kurshelnycky and Reimer (1998) found that Maxforce was highly preferred in bait preference tests, and especially well consumed during the spring and summer. This probably correlates with the colony cycle when egg production and larval growth require large amounts of protein (Reierson et al. 1998).

This preference for protein when brood is present has been shown with *Pheidole megacephala* and *Ochetellus glaber* (Cornelius and Grace 1997).

### **Chapter 3: Outdoor Baiting Versus Spraying for Ant Control**

Perimeter treatments with organophosphates or synthetic pyrethroids are commonly used by pest control operators to control Argentine ants around the outside of structures (Knight and Rust 1990). With this method of insecticide application, label rates require as much as 10 gallons of spray per 1000 ft<sup>2</sup>. Such a strategy does not eliminate or suppress ants but is intended to create a barrier to prevent their entry into a structure. Achieving this goal is difficult because any small gaps or breaks in the barrier are potential passageways, and at best, residual deposits of insecticide can only last ~30 d due to chemical degradation and environmental factors such as high temperature (Rust et al. 1996). In addition, perimeter sprays are usually broad-spectrum insecticides that kill many of the beneficial insects and therefore may result in secondary pest outbreaks (Smith et al. 1996).

One alternative to the use of perimeter spray treatments for ant control is insecticidal baits. Both protein and sucrose-water baits have been shown to be attractive to Argentine ants (Klotz et al. 1998, 2000). The purpose of combining a protein-based granule with a sucrose-water bait is to provide a more complete diet for the Argentine ants. In studies of the seasonal feeding preference of Argentine ants, Rust et al. (1997) found that Maxforce blank was nearly as well taken as 20% sucrose-water. Insecticidal baits are fairly target-specific and use less insecticide per unit area especially if contained within stations. Consequently the use of bait stations is safer for the environment and non-target organisms than application of a perimeter spray. A point resource such as that offered by a containerized bait station also capitalizes on the social behavior of ants whereby scout ants recruit nestmates to a newly discovered food and these recruited ants return to a nest to share this resource with the rest of the colony. Thus baits eliminate the necessity of finding the nest which is usually a labor-intensive procedure due to the cryptic nesting habits of many pest ants.

The objective of this study was to compare the efficacy of bait and spray applications of insecticides for outdoor treatment of Argentine ants, and to determine if a baiting strategy for ants is a practical approach for controlling ants in urban environments.

## Materials and Methods

Test sites consisted of nine private homes located in the cities of Moreno Valley or Riverside, and three buildings on the campus of the University of California, Riverside (all in Riverside County, CA). Each site received one of three treatments: 1) a perimeter spray application of pyrethroid insecticide; 2) baits delivered in stations; or 3) untreated controls. Controls were located on campus sites because homeowner cooperation in the study was contingent upon their receiving some sort of insecticide treatment to reduce their problem with ants.

Estimates of ant activity for each site were based on consumption of 50% sucrose-water (wt/vol) delivered in 15 ml polypropylene monitoring tubes (Falcon Brand conical tubes (17 x 120 mm), Fisher Scientific, Pittsburg, PA) (Reiersen et al. 1998). Each tube contained 12 ml of 50% sucrose-water, more than would usually be collected by the ants in one day. To prevent spillage, but allow feeding, the tubes were placed at an angle on plaster of Paris pedestals and covered with clay pots (15.5 cm diam.) for protection. The tubes were placed 7 m apart around the outside perimeter of the houses and campus buildings, next to the foundation, and left in place for 24 h. Loss of weight from the tubes was corrected for evaporation of liquid and weight of drowned ants. Based on laboratory studies *L. humile* workers can imbibe an average of 0.3 mg sucrose-water per visit. Using this average consumption weight of an individual ant and the adjusted weight loss of the monitoring tubes the number of ant visits per tube over a 24 h period was calculated. This monitoring procedure was conducted before treatment to assess population density at each of the twelve sites, one week after treatment and then bimonthly for 10 weeks. Toxic baits were removed during the monitoring procedure.

Bait station treatments consisted of a 1% hydramethylnon granular protein-based bait (Maxforce Fine Granule Insect Bait, Chlorox Co., Pleasanton, CA) and a liquid bait containing 25% sucrose-water and 0.5% disodium octoborate tetrahydrate (TIM-BOR, U.S. Borax, Valencia, CA). On the eighth week the 0.5% borate was replaced with 0.0001% fipronil (Rhone-Poulenc, NC) as the liquid bait toxicant.

For 1% hydramethylnon delivery we measured 6.2 g of granules into a B&G Perimeter Patrol System (B&G Equipment Co., Plumsteadville, PA) and placed each one on the ground at the same locations as monitoring tubes. Liquid bait stations were placed adjacent to the B&G

stations and consisted of 230 ml acrylic tubes with a matrix of evaporative Coolpad (Research Products, Phoenix, AZ) placed inside a polyvinyl chloride pipe sleeve (Klotz et al. 1998). Bait stations were cleaned and refilled on a weekly basis throughout the 10 week duration of the test.

Perimeter spray treatments consisted of 0.03% deltamethrin (Suspend SC, AgrEvo Environmental Health Inc., Montvale, NJ ) applied in a 1.8 to 2.4 m wide band of spray around the exterior of each residence. The band extended 0.5 m vertically and 1.5 m horizontally. Spray was also applied in a band 1.5 m wide along the margins of sidewalks and pathways and around stepping stones, bases of trees, potted plants, and garbage areas (Rust et al. 1996). Applications were made using a 50-gal (189 L) FMC truck-mounted power sprayer (FMC, Ag Marketing Division, Jonesboro, AR) at the rate of 10 gal (37.9 L) per 1000 ft<sup>2</sup> (92.9 m<sup>2</sup>) measured with an electronic flowmeter (Model PHL-1 Technology Management, Kalamazoo, MI).

**Statistical Analysis.** To reduce variability, post-treatment ant visits were expressed as percents of the pretreatment counts, transformed by  $\log_{10}(x + 1)$  and analyzed by analysis of variance (SAS Institute 1989).

## **Results and Discussion**

The number of ant visits to monitoring tubes in the pretreatment survey were 9170, 22262, and 27297 for the control, spray, and bait sites, respectively. At homes treated with either the bait or spray applications of insecticides, Argentine ant populations were reduced within the 1st week after treatment and remained significantly lower than controls for the 10 wk duration of the test (Table 2). However, foraging activity of ants continued at a low level indicating suppression of foraging workers but not elimination of colonies.

In previous studies using 0.5% boric acid sucrose-water bait stations alone, similar reductions in Argentine ant populations were observed, except the response was slower (Klotz et al. 1998). In the current study the liquid bait was used in combination with a solid protein-based hydramethylnon bait. The rapid reduction of ants in the first week after treatment (Table 2) is probably due to consumption of the hydramethylnon bait. The relatively fast activity of hydramethylnon baits on Argentine ants was noted in an earlier study (Klotz et al. 2000).

The replacement of borate in the sugar water baits with 0.0001% fipronil in the 8th week

of the study, reduced the ant populations in the bait treated homes to their lowest level on the 10th week, yet not significantly different from the spray treated homes (Table 2). Similar to the 1% hydramethylnon solid bait, an ultralow dose of liquid fipronil bait (0.0001%) is relatively fast-acting in comparison with 0.5% liquid borate.

This study demonstrated the long term effectiveness of a thorough spray treatment to control Argentine ants. One application with 0.03% deltamethrin provided 10 weeks of control. The spray application was more extensive than a standard perimeter treatment and included areas where the ants were nesting and trailing as well as around the foundation. The average volume of spray applied at each site was 74.6 liters.

Baiting was ultimately as effective as spraying but it took longer to obtain control and the baits had to be maintained on a weekly basis. This is a heavy investment of time and energy for a pest control operator. Given the current costs for Argentine ant control, this type of baiting program would be cost-prohibitive for a pest control business unless homeowners would be willing to pay more for the less toxic, more environmentally safe approach.

#### **Chapter 4: Toxicity and Repellency of Borate-Sucrose Water Baits to Argentine Ants**

Boron containing compounds such as borax (sodium tetraborate decahydrate) and boric acid have been used since the early 1900s against ants (Rust 1986). Our studies with sucrose water containing boric acid for control of household ant pests have demonstrated that at low concentrations (<1%) boric acid is slow-acting and nonrepellent thereby enhancing long-term ingestion (Klotz and Moss 1996, Klotz et al. 1997). The delayed activity of boric acid promotes a thorough distribution of the active ingredient within the nest, leading to death of the entire colony (Klotz et al. 1996, Stringer et al. 1964).

Commercial ant baits with boric acid or borax as an active ingredient typically use concentrations of 5%. For example Niban (Nisus Corp., Rockford, TN), and Bushwhacker (Bethurum Research and Development, Inc., Galveston, TX) granular baits use 5 and 17% boric acid respectively, and Terro Ant Killer II (Senoret Chemical Co., Inc., Kirkwood, MO) liquid bait uses 5.4% borax. The new liquid ant baits being developed use much lower concentrations of boric acid. Drax Liquidator (Waterbury Companies, Inc., Waterbury, CT), Dr. Moss's Liquid Bait System (JT Eaton & Co., Inc., Twinsburg, OH), and Advance Liquid Ant Bait (Whitmire

Micro-Gen, St. Louis, MO) use 1% boric acid in sucrose water. Another borate, disodium octaborate tetrahydrate has been formulated at 1% in a sweet liquid bait, VG AB 3 (EPA registration pending) for use against carpenter ants (Wegner 1998).

Our objectives in this study with Argentine ants were to 1) compare the oral toxicities of low concentrations of boric acid, borax, and disodium octaborate tetrahydrate; and 2) test for a feeding preference between disodium octaborate tetrahydrate and boric acid and for different concentrations of boric acid. Results of this study may help optimize development of boron liquid bait formulations for controlling ants.

## **Materials and Methods**

**Toxicity Tests.** Argentine ants were collected from a citrus grove on the Riverside campus, University of California (Riverside County). The ants were provided water but no food for 1 d prior to bait exposure. Concentration-mortality was determined with procedures described by Klotz et al. (1998). Crystalline boric acid and anhydrous borax (99% [AI]; Sigma, St. Louis, MO), and TIM-BOR (98% disodium octaborate tetrahydrate; U.S. Borax Inc., Valencia, CA) were dissolved in 25% (wt:vol) sucrose-deionized water solutions to produce two concentrations (0.5 and 1%) of each boron compound. Bait solutions were added to cotton plugs daily inside small petri dishes with 10 ants. Treatments and controls (25% sucrose-deionized water) were replicated five times. The bait solutions were available continuously to the ants for the duration of the test. Daily observations on cumulative mortality were recorded for 5 d.

**Preference Tests.** Binary choice tests were designed to determine feeding preferences of the ants on different solutions. The consumption of solutions delivered to the ants in side-by-side feeding stations attached to trees was used to measure preference. Feeding stations were constructed from 50-ml capped centrifuge tubes by drilling a 2-cm-diameter hole through a 3.2-cm-diameter cap. A 4.5-cm square of WeedBlock (Easy Gardener, Waco, TX), a porous plastic material through which the ants could drink, was centered over the top of the centrifuge tube filled with solution and the cap screwed down to secure it in place. The bait stations were inverted and taped at eye level to the trunks of trees containing foraging trails of Argentine ants. To test their preference between 1% disodium octaborate tetrahydrate and 1% boric acid (both in 25% sucrose water) tubes of each solution were taped next to each other on each of 15 trees.

A similar test with 10 trees each was conducted for sodium (Na) and pH preference in solutions containing equal concentrations of boron. When disodium octaborate dissolves in water the following reaction occurs:



Because of the differences in molecular weight a 1% (10g/l) solution of disodium octaborate tetrahydrate is equivalent to a 1.2% boric acid solution with 1.9g/l of NaOH added. Boric acid solutions were prepared with NaOH and NaCl, to equal the boron and Na<sup>+</sup> content of 1% disodium octaborate tetrahydrate solutions. All solutions were prepared in 25% sucrose water. The boric acid solution with NaCl (2.8g/l) was used to study the relative effects of Na<sup>+</sup> and pH preference. This solution had a pH that was similar to the boric acid solution (?4.7), but had the same Na<sup>+</sup> concentration as the solution with NaOH. The boric acid + NaOH solution had a pH and Na<sup>+</sup> concentration that were similar to the 1% disodium octaborate tetrahydrate (?7.7).

To test for discrimination (preference test) of different concentrations of boric acid in sucrose water, each boric acid solution (0.5, 1.0, 2.0, and 4.0%) was paired with a control containing only sucrose water. Each concentration was replicated on 10 trees. All test solutions and controls used in these studies contained 25% sucrose in deionized water (wt:vol).

The ants were allowed to feed on the various solutions for at least 24 h after which the amount consumed by the ants in each of the vials was recorded. Consumption was determined gravimetrically with 1% boric acid and disodium octaborate tetrahydrate. Consumption was determined volumetrically in the other preference tests and standardized in milliliters per hour by dividing the total volume consumed by the number of hours the ants were allowed to feed.

**Statistical Analysis.** Mortality data were corrected with Abbott's (1925) formula and analyzed by probit analysis (Raymond 1985) to determine lethal times (LT<sub>50</sub>) for each concentration. The value of  $c^2$  was used to measure the goodness of fit of the probit regression line. Binary choice tests to determine feeding preferences of ants for different solutions were analyzed using a paired *t*-test comparison (StatView 1992). Regression analysis was performed on LT<sub>50</sub>s of the different borates as a function of boron concentration (Grapher 1996).

## Results and Discussion

The time required to kill 50% of the workers of *L. humile* (LT<sub>50</sub>) decreased with increasing concentration of the three boron compounds, indicated by nonoverlapping 95% confidence limits (Table 3). Anhydrous borax, boric acid, and disodium octaborate tetrahydrate are 21.5, 17.5 and 21% boron by weight, respectively. When the compounds are expressed in boron equivalents, the LT<sub>50</sub> values are a function of the boron concentration (Fig. 4). The log-linear relationship between LT<sub>50</sub> and boron concentration for the different compounds was highly significant ( $P < 0.01$ ) with an  $R^2 = 0.94$ .

Based on the consumption rates of 1% boric acid and disodium octaborate tetrahydrate in sucrose water placed side-by-side in the binary choice test, we could detect no preference (i.e., no significant difference) by the ants for either solution [paired  $t$ -test = 1.22,  $df = 14$ ,  $P = 0.24$ ]. When the pH is made approximately equivalent for the two solutions by the addition of NaOH, no preference was shown by the ants for either solution [paired  $t$ -test = -1.46,  $df = 9$ ,  $P = 0.18$ ]. Similarly, no preference was shown for solutions with different pH but equal Na<sup>+</sup> concentration when NaCl was added to the boric acid solution [paired  $t$ -test = -0.96,  $df = 9$ ,  $P = 0.36$ ]. Although there is no toxicological difference or feeding preference between boric acid and disodium octaborate tetrahydrate that would make one better than the other as an active ingredient, one advantage to disodium octaborate tetrahydrate, which is hydrated, is that it dissolves rapidly in water making it easier to formulate water soluble baits. All solutions made from boric acid or borate salts have in common the boron existing as uncharged boric acid molecules at pH < 8.

Consumption of sucrose water was significantly higher than consumption of the >1% boric acid solutions indicating unpalatability of boric acid at 2 and 4% (Table 4). Likewise, in a consumption test conducted in the laboratory with *Solenopsis invicta* Buren, feeding on solutions of 0.25, 1, and 5% boric acid in 10% sucrose water there was a significant reduction in the amount of the 5% solution consumed (Klotz et al. 1997). Therefore, to avoid repellency in liquid baits with boron compounds as an active ingredient formulations should use ?1%. Although there is little information available concerning the physiological mode of action of borax and boric acid on insects (Rust 1986), it has been shown that borate ions form strong complexes with sugar alcohols, such as inositol, and other organic functional groups (Hu et al. 1997, Williams and Atalla 1981, Woods 1994). In addition, boron may be involved in the disruption of intercellular adhesion since saturated boric acid solutions can be used to dissociate

cells (Goodrich 1942).

## **Chapter 5: Argentine Ant Trail Pheromone Enhances Consumption of Liquid Sucrose Solution**

Wilson and Pavan (1959) and Robertson et al. (1980) showed that artificial trails from ventral organs (Pavan's gland) of the Argentine ant, *Linepithema humile* (Mayr), elicited trail following by workers of this species. Cavill et al. (1979, 1980) identified Z9-16:Ald as the behaviorally active compound in these glands. Van Vorhis Key et al. (1981) and Van Vorhis Key and Baker (1982) measured response rates to the pheromone and showed that Argentine ants will follow synthetic trails. They demonstrated that airborne trail pheromone could be detected and followed. Van Vorhis Key and Baker (1986) concluded that Z9-16:Ald is the most important component of the trail pheromone, although other synergists are possible. The synthetic form was 100% less active than the natural compound (Van Vorhis Key and Baker 1982).

Van Vorhis Key et al. (1981) showed that ants could follow an airborne trail up to 6 mm away from its source. Furthermore, trail following diminished as the pheromone concentration increased beyond 1 ant equivalent of extract per 50 cm of trail. These results suggest that at higher concentrations the pheromone may be repellent. They also showed that on filter paper, activity declined to half the original level after 24 h and was almost gone completely after 8 h. Workers would follow trails longer if they had a previous encounter with a recruiting ant (Van Vorhis Key and Baker 1982).

Given these findings, we address the question of whether the synthetic trail pheromone can increase worker recruitment to liquid food sources, thereby enhancing consumption rates. Rather than attempting to describe which aspects of recruitment behavior are involved, we are using the word "recruitment" generically to refer to an increase in the number of feeding ants. This study was not designed to elucidate the mechanisms responsible for the observed behavior. An inexpensive form of the pheromone is commercially available, facilitating experimentation. As baiting technology advances, the use of natural products to enhance baits is a fertile area for research.

### **Materials and Methods**

Argentine ant colonies were kept in the laboratory in plaster of Paris nests inside a plastic shoe box (36 by 30 by 13 cm). Each colony contained several thousand workers and several queens. They were fed a diet of 10% sucrose water and insects. The nest box was connected by a flexible plastic rod (4-mm diameter) to another 44 by 56 by 12 cm plastic tray that served as a foraging area. Both the box and tray were coated with Fluon (Imperial Chemical Industries Americas, Bayonne, NJ) to prevent ant escapes. We did all laboratory pheromone tests on glass slides placed in the foraging box. Thus, foragers had to cross back over the connecting bridge to recruit workers. The slides were thoroughly cleaned with soap and water and rinsed with acetone between trials. Ants were provided water but no food for 1 d prior to tests.

The synthetic trail pheromone Z9-16:Ald was ordered from the Aldrich Chemical Co., Milwaukee, WI. The pheromone has the appearance of a viscous oil at room temperature. We dissolved it in hexane and then prepared serial dilutions, as described below.

**Laboratory Tests.** We prepared serial dilutions of the trail pheromone varying by 1 order of magnitude over the range of 0.01 to 100  $\mu\text{g}$  of pheromone per milliliter of hexane solution. We put a 20- $\mu\text{l}$  drop of each solution onto a depression slide and mixed it with 50  $\mu\text{l}$  of 10% sucrose solution. After the hexane evaporated, the treated slide and a 2nd slide with only 10% sucrose solution were placed side by side in the foraging areas of 3 Argentine ant colonies. Every 3 min during a 30-min period we counted the number of ants feeding at the sucrose solution on each slide. In all, 21 paired choice tests were run over the 5 pheromone test-concentrations.

As a control we also ran 9 paired choice tests where 1 slide contained 50  $\mu\text{l}$  of a 10% sucrose solution and the adjacent slide contained 20  $\mu\text{l}$  of hexane (without the pheromone) plus 50  $\mu\text{l}$  of the same sucrose solution.

We did statistical tests at each pheromone concentration by using a mixed model ANOVA where each of the 3 colonies represents a block (the random effect). Each block consisted of 10 paired measurements taken over the 30-min test period. The treatment effect was tested using interaction as the error term (Systat 1997).

**Field Tests.** Field tests were conducted during October and November 1997 at a citrus grove on the University of California, Riverside, campus. We selected trees with active ant trails

going up the trunks. The 10% sucrose solution was poured into 50-ml vials. Using the results of the laboratory tests as a guide, we pipetted 100  $\mu$ l of a 10- $\mu$ g/ml hexane solution onto a plastic membrane that contained evenly spaced 1.5-mm holes across its surface (Vispore Polymer Fabrics #6607, Tredegar Film Products, Richmond, VA). After the hexane evaporated, these membranes were placed over the vials and held in place by a cap that had its center removed to expose the membrane. When the vials were inverted, ants could drink from the small holes in the membrane. One control and 1 pheromone vial were taped side by side to each of 23 trees for 24 h, beginning at 1200 hours (PST). In a 2nd series of trials 12 pairs of vials were left outside for 24 h. Consumption was measured by weighing vials before and after the tests and converting to milliliters. We evaluated the results for significance using the paired *t*-test (Systat 1997).

We also did 11 paired comparisons as a hexane control by repeating the above experiment (8 trials were done over 4 h and 3 trials over 24 h) and applying just hexane on one of the membranes instead of a mixture of hexane and pheromone. The results were evaluated with the paired *t*-test.

## Results

**Laboratory Tests.** Fig. 5 defines and illustrates the percentage of feeding enhancement (recruitment) over the experimental range of pheromone concentrations. Both the 10  $\mu$ g/ml and the 1  $\mu$ g/ml concentrations had a significantly higher number of feeding ants ( $F = 45.7$ ;  $df = 1, 3$ ;  $P = 0.007$  and  $F = 10.8$ ;  $df = 1, 4$ ,  $P = 0.03$ , respectively) than the controls. The pheromone ceased to attract significantly more workers at 1 order of magnitude above the optimal concentration of 10  $\mu$ g/ml. Although the controls and treatments at the lower concentrations were not significantly different due to their small sample sizes, even the lowest pheromone concentration showed some enhanced recruitment. The photograph inserted in Fig. 5 shows the enhanced recruiting effect of the pheromone when mixed with the sucrose solution. The ants did not significantly prefer the hexane sucrose solution control over the plain sucrose solution ( $F = 0.68$ ;  $df = 1, 8$ ;  $P = 0.43$ ).

**Field Tests with Liquids.** Within minutes of taping vials to the trees more ants were observed on the pheromone-treated plastic membrane. The same was true after 4 and 24 h. Many

ants started to drink immediately upon encountering the sucrose solution. In the 4-h trials mean consumption rate increased 29%. For the 24-h field trial there was a 33% increase in the consumption rate (Table 5). The 24-h trial had a lower consumption rate overall than the 4-h trial because they ran overnight; in the fall nighttime temperatures are low enough to reduce foraging. In the controls investigating the effect of hexane alone, consumption of sucrose solution did not differ significantly among the paired vials (Table 5).

## **Discussion**

We report on a successful and economically feasible way to enhance Argentine ant recruitment to liquids by combining them with pheromones. Hölldobler and Wilson (1990) tabulated 63 ant species known to produce trail pheromones; 16 of the pheromones have been identified, yet few attempts have been made to combine these compounds with food for possible use as bait. Robinson et al. (1982) tested several leafcutter ant species [*Atta sexdens rubropilosa* Forel, *Atta cephalotes* (L.), and *Acromyrmex octospinosus* (Reich)] by adding their trail pheromone to soybean [*Glycine max* (L.) Merr.] baits. Only 1 of their trials with 1 species (*A. octospinosus*) showed increased bait collection in the field, but the increase was not consistent. One apparent problem was that if another attractant, such as sugar, was already on the bait, the effect of the pheromone was lessened. Robinson et al. (1982) concluded that addition of the trail pheromone would not be a cost-effective bait treatment for these ants.

Another attempt to enhance baits with trail pheromones was made by Vilela and Howse (1988) with the leafcutting ant *A. sexdens rubropilosa*. They used whole abdominal extracts applied to vermiculite particles and found an increase in the number of particles removed from their original location, although most were not taken to the nest. However, in field tests with the extracts applied to citrus-pulp bait, removal rates were not improved.. Vilela and Howse (1988) found that although the pheromone attracts ants, it does not necessarily increase bait removal.

Other pheromones also can have attractive properties. In red imported fire ants, *Solenopsis invicta* Buren, Vander Meer et al. (1980) showed that the poison sac was the source of a queen pheromone. Lofgren et al. (1983) showed that this pheromone attracts workers when applied to rubber septa or when carried by an airstream into an olfactometer. It has not yet been shown whether these attractants can be used successfully in ant baits.

In Argentine ants, Baker et al. (1985) showed that workers preferred 25% honey or sucrose

water over granulated sugar or other solid food containing proteins. Our results indicate that the trail pheromone, if on or near a desirable food (e.g., the sucrose solution), increases the number of feeding ants. Given the negligible cost of the pheromone (?\$16/100 mg), it could be included with liquid baits designed for Argentine ants.

## **Chapter 6: Chemical and Physical Barriers to Argentine Ants**

Chemical signals dominate the communication systems of social insects such as ants (Wilson 1971). For example, the Argentine ant, *Linepithema humile* (Mayr) (= *Iridomyrmex humilis*), produces a trail pheromone from the sternal gland (Wilson & Pavan 1959). Over evolutionary time, ants, other insects and plants have developed chemical methods of defending themselves against ant attack (Hölldobler & Wilson 1990). We may be able to exploit these same chemical defenses in management of pest ants. Progress in the use of repellents in pest ant management has gone farthest in agriculture, where research has focused primarily on plant protection (Shorey et al. 1992, 1993, 1996). For example, repellents applied in a band around the trunks of citrus trees can prevent Argentine ants from tending honeydew-producing homopterans. Candidate chemicals are placed on cotton twine or rubber tubing and then wrapped around tree trunks; the number of ants crossing this potential barrier are counted to evaluate a compound's repellency (Shorey et al. 1992). In Table 6 the efficacy of the repellent farnesol with the carrier Stickem is evaluated against a Lorsban (chlorpyrifos) spray. Farnesol-Stickem remains effective as a barrier for 5 mo. Other carriers for repellents have been investigated, but the best so far has been Stickem Special (Seabright, Emeryville, California), which shows some repellency of its own (Table 6) (Shorey et al., unpublished data).

To identify potential repellents, food sources may be set out in a laboratory foraging arena and surrounded by each chemical compound to be evaluated as barriers to foraging ants (Shorey et al. 1996). In this type of assay, farnesol provides an effective barrier against Argentine ants.

Repellents may have a place in urban settings for management of ants in orchard or shade trees around homes, and in sensitive areas like hospitals, animal rearing facilities, computer equipment, vending machines, food processing and storage facilities. If repellents could be formulated in sprays for perimeter treatments, they would be an ideal way to pestproof homes prone to ant infestation. Or, used in concert with baits, repellents might alleviate the immediate

problem of ants entering a house while allowing time for slower-acting baits to take effect on the outside.

In a test of enhancing consumption of bait using a sticky barrier, which blocked the ants travel into the tree canopy, significantly more bait was consumed in trees treated with Stikem versus those without. On the trees where we used Stickem as a barrier the consumption of bait was twice that of trees without a barrier (Fig. 6). This approach might be incorporated into a management strategy, whereby these barriers would prevent the ants from tending aphids in the trees and shrubs, cutting off their food supply, and directing the ants to baits, thereby speeding up the control process.

The strategies emphasized in this research are low-toxic and nonchemical. These techniques do not provide the quick-fix that many demand from pest control. That kind of solution can be obtained with some of the broad-spectrum contact insecticides. Our goal is to offer an IPM approach to ant control.

Future development of alternative strategies for household ant control depend on the commitment of both industry and academia to support research on innovative techniques that would significantly reduce the risk of exposure to pesticides and offer an environmentally-safer alternative for ant control.

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