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Laboratory and field techniques for development and evaluation of a bait for urban ant pests

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ABSTRACT
The pest control industry is currently developing new baits for urban and industrial pest management of ants. Recent safety and environmental concerns with pesticide use has renewed the public's interest in the least toxic control of these pests. Our studies with several species of urban pest ants demonstrate that a low concentration of boric acid bait mixed with sugar and water is an effective slow-acting toxicant. Some of the advantages of this bait include the delayed toxicity and water solubility of boric acid at low concentrations. Additionally, the water carrier and sugar attractant meet the requirements of ants for moisture and carbohydrates.

INTRODUCTION
In a recent national survey in the U.S.A., ants were considered by homeowners to be a more serious household pest than cockroaches (1). Along with this growing economic concern for urban pest ants, there is renewed interest in the development of baits for their control. This new popularity of baits is probably due to several factors including public pressure to reduce pesticide use, current availability of insecticides ideal for baiting ants, and numerous advantages which baits confer to pest control (2). Baits are more target specific than more traditional techniques of ant control which have relied on applications of insecticides, placing heavy loads of broad spectrum insecticides into the environment (3). Baits are more cost effective compared to labor intensive inspections to locate and destroy nests, and treatment strategies involving invasive techniques like drilling and dusting structural voids. And, if used properly they are highly effective: they exploit the natural foraging behavior of ants to recruit and share resources, thereby spreading the bait toxicant throughout the entire colony, and eventually destroying it (4, 5).

On the negative side, baits tend to be slow-acting, requiring that a homeowner, for example, be educated on how baits work and the length of time required to gain control. Also, their shelf-life can be limited by the food contents. However, we feel the advantages of

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baits far outweigh these disadvantages which can be alleviated through education of the public and pest control industry and new and improved bait formulation.

A bait consists of four components (6): 1. an attractant, usually a food or pheromone which makes the bait acceptable and readily picked up (7), 2. a palatable carrier, which gives the physical structure or matrix to the bait, 3. a toxicant, which should be non-repellant, and delayed in action, effective over at least a ten fold dosage range (8), and 4. other materials added for reasons of formulation, such as emulsifiers or antimicrobial agents. Each of these components must be developed and tested for efficacy.

In recognition of the increased interest in bait development for ant control, our goal here is to present our laboratory and field methods as model approaches to the evaluation and performance of baits. Our methods, as well as those of other investigators, can be found in other publications. Here we bring together in one place our techniques as a basis for comparison with the procedures of other workers, and present sample data for each procedure to illustrate key points in the analysis and interpretation of results.

To achieve this end we will focus on a boric acid sucrose and water bait using the black and Florida carpenter ants, *Camponotus pennsylvanicus* (DeGeer) and *C. floridanus* (Buckley), the red imported fire ant, *Solenopsis invicta* Buren, and the Pharaoh ant, *Monomorium pharaonis* (L.), as our examples.

MATERIALS AND METHODS

In bait testing and evaluation of bait performance, we offer the following procedures as models for experimental design and analysis of results. In the first procedure, we are conducting a bait acceptance test to evaluate the attractance of different sugars to *C. pennsylvanicus*.

Procedure 1: Sugar acceptance tests conducted in the field with *C. pennsylvanicus*.

Experimental design. We conducted these studies at night because *C. pennsylvanicus* is primarily nocturnal (9). We used carpenter ant colonies nesting in live trees, where we set up a series of 3-cm-diameter wooden poles arranged in a horizontal runway, supported by stakes at ≈50 cm above the ground. The runway extended away from the tree trunk, to a distance of 1.5 - 5.0 m where it connected to a vertical dowel which led the ants up into an arena (90 cm diameter) through a 3 cm diameter hole drilled in the center of the arena floor. We enticed foraging ants to travel the length of the runway and up into the arena, by placing a 150 cm square feeding station on the pole adjoining the tree and providing the ants with freshly diced insects (*Tenebrio molitor* (L.) larvae, *Blatella germanica* (L.), or both) and sugar-milk (1:3) in shallow, 2 cm diameter dishes. After the ants had discovered this food source and recruitment of nestmates had begun, we periodically moved the station a little farther away from the tree until finally it was located on the floor of the arena. After the ants were accustomed to the arena, we placed the various sugar baits in a circular array (30 cm diameter) within the foraging arena, and removed the feeding station. After a single evening of foraging on the baits in this arena, the ants no longer required further training on succeeding nights, since they showed up in the arena before bait testing even began. We weighed baits in the laboratory before placement in the arena, and reweighed them the following morning after the foraging ants had fed upon baits in the test array. We made weight change corrections to adjust for evaporation.

On each of 3 nights we ran a replicate of the baits on 3 different colonies. For each test we positioned the baits in the arena differently by using a random numbers table. We quantified collection of bait to determine preference, by calculating the percentage of each bait collected of the total of all baits collected. We did this to adjust for possible differences in colony size, activity, as well as weather conditions which might vary from night to night.

Bait preparation. We prepared 1 M solutions of the refined sugars in distilled water. We diluted the natural sugars in 50 ml distilled water using 17.11 g honey and 17.11 g raw sugar (this dilution based on 1 M sucrose dilution). We prepared all refined sugar gel baits by adding 140 mg of Gelgard™ (product of Dow Chemical Co., Midland, Mich.) to 50 ml of the sugar solutions. The natural sugar gel baits were prepared with 280 mg Gelgard added to 50 ml solutions; this greater quantity of Gelgard was necessary to approximate the viscosity of the gel baits prepared from the refined sugar solutions. After preparation, the baits were stored at room temperature in glass beakers sealed with Parafilm™. For testing, a 5 gm portion of gel bait was poured into a small, plastic petri dish which was transported to the field within two hours of being dispensed from the bulk preparation.

Statistical Analysis. We analyzed the percent bait collection using a mixed model two factor ANOVA (randomized block design), Yij = u + Bi + Cj + Bcij + e(ij)k, with the percent weight of bait removed being the main factor (B) and colony a random blocking factor (C). If, as in most cases, there was no colony effect, we dropped this factor and the examination of bait preference was based on pooled data from the three colonies and three replicate nights (n = 9). We did not include night as a factor since relative bait collection rather than absolute bait collection was the value of interest. We used mean separation with Least Significant Difference (P = 0.05 level) as an a posteriori multiple comparison of means. SAS procedures (10) were used for all analyses.

After identifying a suitable sugar for the bait, our next step was to choose an appropriate toxicant. Our decision was based on our choice for the carrier, water. Other ant baits use different carriers, for example soybean oil in some of the fire ant baits. For these baits, oil soluble compounds are used as toxicants. For our bait we chose a water soluble compound, boric acid. In the following procedure we conduct oral toxicity tests to obtain a dosage response of the ants to boric acid.

Procedure 2: Primary toxicity tests conducted in the laboratory with worker ants of *C. Floridanus* (11).

We collected carpenter ants in Alachua County, Florida, using the portable vacuum method of Akre et al. (12). In the laboratory (25°C, ambient RH) we provided the ants with water but no food. One day post-collection, we chose medium size worker ants out of the colonies to achieve uniformity of size for the oral toxicity tests. We distributed them, 10 each, into plastic petri dishes (145 X 25 mm, Thomas Scientific) each supplied with a scintillation vial (7 ml, Kimble) plugged with cotton in which we dissolved crystalline boric acid (Sigma Chemical Co., St. Louis, MO, 99% AI) in a 10% (wt/vol) sucrose-deionized water solution to produce various percent concentrations (0.13-3.13) of boric acid. Ants fed immediately on the baits as evidenced by their abdominal distension within several hours after the tests were set up. We replicated treatments and controls (sucrose-deionized water) on successive days and we used ants from a different colony for each replicate. The bait solutions were available continuously to the ants for the entire duration of the test. Then, we placed the ants in a climate-controlled chamber, held at 27 °C and 80% RH. We made daily observations on cumulative mortality and recorded these for seven days. We corrected mortality data with Abbott's (13) formula and analyzed by probit analysis (14) to determine lethal time (LT_{50}) values for each concentration of boric acid.

After obtaining the dosage response of individual worker ants to the bait, we then chose several concentrations of boric acid in the bait to test its effect on large queenright colonies.

Procedure 3: Secondary toxicity tests conducted in the laboratory with colonies of *S. invicta*.

We conducted laboratory tests using queenright colonies which were approximately 10-20 months old. Each test colony initially contained 60,000--75,000 workers and 60--70 ml of brood (eggs, larvae and pupae). We reared colonies according to methods described by Banks et al. (15), except the diet consisted of 25% honey-water, crickets and hard-boiled chicken eggs. We withheld food from the colonies for 1 d prior to treatment. We prepared the boric acid - sucrose solution in the same manner as described above for the oral toxicity tests. We tested four different concentrations (0.25, 0.50, 0.75 and 1.00%) of boric acid bait. The control bait consisted of 10% sucrose-deionized water. We offered liquid bait (72 ml) to each laboratory colony in 200- by 25-mm test tubes plugged with cotton. We provided water continuously and ants were allowed ad libitum feeding on the bait, crickets and eggs for the duration of the test with baits replaced every 2 wk. We observed worker mortality and queen status (dead or alive) weekly. We determined brood reduction by visually comparing a photograph of known quantities of brood with the brood present in a colony. We monitored colonies until it was noted that the queen either died or was small in size and not producing eggs, brood was absent and there was at least a 99% reduction of workers. We used three colonies for each concentration of boric acid and the control. We determined treatment efficacy by the percent reduction in a population index (PI) (16). To determine the PI, we rated each colony before treatment and at weekly intervals after treatment. With this method, we assigned colonies a rating of 1 to 6 based on colony size (number of workers ranging from <100 workers to >50,000) and 1 - 25 on the basis of quantity of worker brood (0 - >30 ml). We then used the products of these ratings to calculate the percent reduction PI for each wk ($PI_{wk\ 0}$ - $PI_{wk\ x}$ /$PI_{wk\ 0}$) x 100.

In these colony tests we noted a possible inverse relationship between bait consumption and concentration of boric acid, so we decided to test this hypothesis in the laboratory.

Procedure 4: Bait consumption tests conducted in the laboratory with colonies of *S. invicta*.

We dissolved boric acid in 10% deionized sugar water to produce solutions of 0.25, 1 and 5% (wt:vol). Controls consisted of 10% sucrose in deionized water. We added each solution (≈50 ml) to test tubes (150- by 25-mm) and plugged with cotton. We starved all colonies for 1 d prior to bait exposure. We provided the boric acid - sucrose solution for 24 h to large monogyne colonies (>50,000 workers) without an alternative food source. We replicated treatments and controls three times. In addition, to correct for evaporative water loss, we ran concurrently three replicates for each of the treatments and control in adjacent nest boxes without ants. We calculated the consumption of bait after 24 h by subtracting the weight of the test tube and bait after the test from the weight of the test tube and bait before the test. We then corrected the resulting difference for evaporative water loss by subtracting the mean of the 3 evaporative standards. We compared mean consumption of toxic baits to the control using ANOVA ($P < 0.05$), and Scheffe's F test (17) for separation of means.

At this point we felt we had enough laboratory information about our bait's efficacy that we were ready to conduct a preliminary field test against a structural infestation of Pharaoh ants.

Procedure 5: Field test of the bait against an infestation of *M. pharaonis* in an apartment complex.

Experimental design. Our study site was an apartment complex located in Gainesville, Florida. We used six single-story buildings (≈176 m^2 interior area per building), each consisting of four one-bedroom apartments. We adopted the experimental design from another bait study conducted at the same location by Oi et al. (18). We estimated size of Pharaoh ant foraging populations by placing white index cards (7.5- by 6.5 cm) baited with honey (≈1 g) at 6 locations inside and 6 locations outside each apartment. Interior card placements were in the living room on the window sill; in the kitchen on the sink counter, and on the wall near the fuse box; in the bathroom on the basin counter, and on the wall in the vicinity of the toilet; and, in the bedroom on the window sill. Exterior locations included the bottom of the front door; the top of the courtyard gate; on top of the courtyard wall at the intersection of the courtyard and apartment walls; and, the courtyard window sill. We selected the remaining exterior locations from the following areas: the water spigot; the wall/air conditioning hose junction; and the electric meters. We placed index cards on vertical wall surfaces using poster putty. We set cards in place between 0930 to 1200 hours EDST and checked them ≈2 h later. We counted Pharaoh ants on each index card and then shook them off at the same location. We suspended the normal pest control service for the duration of the study.

We conducted an initial ant survey using bait cards as described above, at the apartment complex on 12 Oct 1995 to determine foraging locations from all buildings. From this initial survey, we chose 6 buildings infested with Pharaoh ants for the study.

To determine the effectiveness of a 1% boric acid sucrose solution in reducing Pharaoh ant populations, we randomly assigned treated and untreated bait stations to the 6 buildings so that 3 buildings were used per treatment and control. We determined pretreatment populations from the survey used in the foraging study reported above. We positioned bait stations adjacent to the index card locations using double-sided tape, on the same day as the pretreatment survey, immediately after counts were made. Stations consisted of small Gelman™ Petri dishes (50 mm dia. by 9 mm h., Fisher Scientific) each supplied with a wad of cotton soaked in 7 ml of either 1% crystalline boric acid (Sigma Chemical Co., St. Louis, MO, 99% AI) dissolved in 10% (wt/vol) sucrose-deionized water solution; or 10% sucrose solution alone which was used as the control. The lid of the petri dish had nine small holes (≈3 mm diam.) to allow ant entry and prevent evaporation. We replaced stations each week with fresh bait for the first 3 wk and then removed them. We monitored post-treatment populations weekly for 7 wk (19 Oct through 15 Dec 1995) following the same procedure as the pretreatment survey. Outdoor temperatures during the population monitoring ranged from 19.0°C to 34.5°C.

Statistical Analysis. The mean number of ants per card for the control and treated buildings were evaluated by the general linear model (GLM) procedures (19) for each sample date. Means were transformed with the log(x+1) to reduce variation and to generate a more normal distribution.

RESULTS AND DISCUSSION

When developing the boric acid sucrose - water bait, our intention was to exploit the natural feeding habits of urban pest ants which forage for honeydew. For ants which are adapted to collect, transport and exchange honeydew by trophallaxis, a liquid bait is ideal. Using water as the bait carrier offered the additional advantage of providing moisture to the ants which in some urban situations can be a limiting factor. Since sugars are a major component of honeydew (20), we decided to first investigate differential feeding preferences for various kinds of sugars.

The results of procedure 1 (Fig. 1) indicated that honey was significantly preferred over all the other sugar baits. Each colony was collecting approximately 1500 mg of honey bait per night. The other natural sugar, raw cane sugar, and sucrose were equally attractive to the ants. Approximately 1000 mg of these baits were collected each

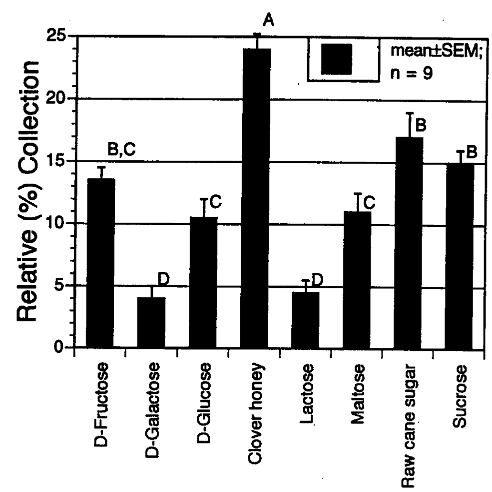

Fig. 1. Bait collection by *Camponotus pennsylvanicus*. Percent collection of various sugars was significantly different by one factor ANOVA (F=27.32, df=7,64, P<0.0001). Means followed by the same letter are not significantly different (P = 0.05; Least Significant Difference).

night. Next in preference were baits containing fructose, maltose or glucose which were equally collected. Lactose and galactose were not very attractive to the ants. Sucrose, then, appeared to be a good candidate for the food component of our bait. It is a constituent of honeydew, plant sap and nectars (21, 22,23, 24), which are attractive foods for many species of ants, and has been shown to have excellent phagostimulant activity for *S. invicta* (25).

Since boric acid is water soluble, it was a likely candidate for a bait toxicant. However, there was no previous evidence for it exhibiting delayed activity, which is a necessary requirement for an effective ant bait. From our dosage response in procedure 2 (Fig. 2) we discovered that at low concentrations boric acid does indeed have delayed action. Over the dosage range from 0.13 - 3.13% boric acid, median lethal times (95% CL) ranged from 9.7 (8.1-13.3) d to 1.5

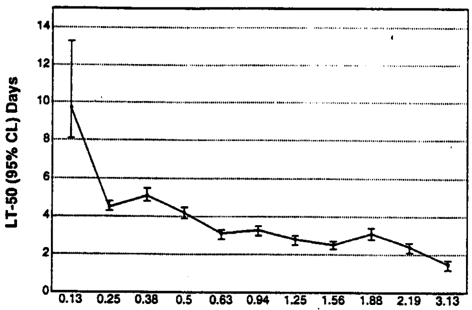

Fig. 2. LT_{50}s (95% CL) of *Camponotus floridanus* workers exposed to various concentrations (0.13-3.13%) of boric acid in a 10% sucrose water bait. For each concentration >70 ants were tested (11).

(1.2-1.7) d. In comparison with boric acid baits developed in the past, we found that the effective concentration could be significantly reduced.

Based on these results we decided to look at the effects of low concentrations of boric acid on large colonies of ants.

In procedure 3, concentrations of boric acid ranging from 0.25 - 1.0% in the sucrose water bait were fed to large colonies of *S. invicta*. After 6 wk of continuous exposure to 0.25, 0.5, 0.75 and 1% boric acid - sucrose water bait, there was a 90% reduction of workers and brood (Fig. 3). By the 16 wk, in all treated colonies there was a 99% reduction of workers, no brood and the queens which remained were small and not producing eggs.

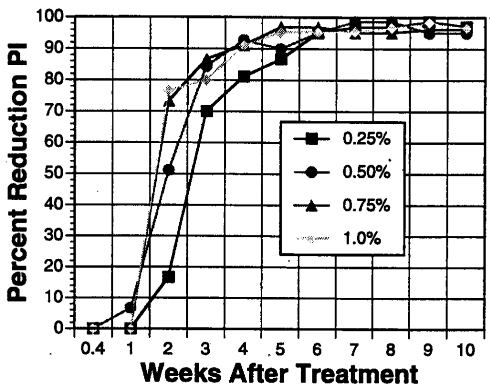

Fig. 3. Mean percent reduction of PI (population index) in *Solenopsis invicta* colonies exposed for 10 wk to various concentrations of boric acid in 10% sucrose water. The PI is a combined value of worker number and brood quantity. Each point represents the mean of 3 colonies of fire ants, each with one queen and >50,000 ants at the beginning of the test.

We noted during the colony tests above that consumption of bait seemed to be inversely correlated with concentration of boric acid. In our test of this hypothesis in procedure 4, we found that consumption of the higher concentration (5%) of boric acid bait was significantly lower than the control (Fig. 4).

Our field test of the bait with Pharaoh ants in procedure 5 was very encouraging. After one week of exposure to a 1% boric acid bait there was a significant reduction of ant activity when compared to the control (Fig. 5). This reduction was maintained for 7 wk. At 8 wk the number of workers counted in the treated buildings was not significantly different from that in the control buildings. The bait, then, reduced the problem below pest threshold but did not eliminate the Pharaoh ants.

The findings from these studies have revealed a potential route for developing highly attractive baits for pest management of several of the urban pest ants. However, to develop this bait further will require that we investigate further refinements. First, the optimal concentration of sucrose to be used in the bait may vary from place to place depending on the ants' dietary preferences. Water stress may also result in differential preferences. Second, because sucrose in water has low volatility, other co-factors might be added to the bait to advertise its presence over distance, thereby attracting ants rather than relying on the ants to find the bait by random search behavior. Third, delivery systems for liquid baits also need to be developed. One possible design consists of a sponge soaked

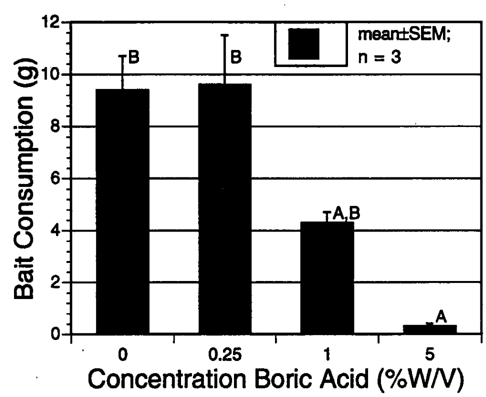

Fig. 4. Consumption of boric acid in 10% sucrose water solutions by *Solenopsis invicta* colonies in 24 h. Consumption was significantly different by ANOVA (F=14.7, df=3,8, P=0.0013) for the various concentrations of boric acid. Means followed by the same letter are not significantly different (P = 0.05; Scheffe's F test).

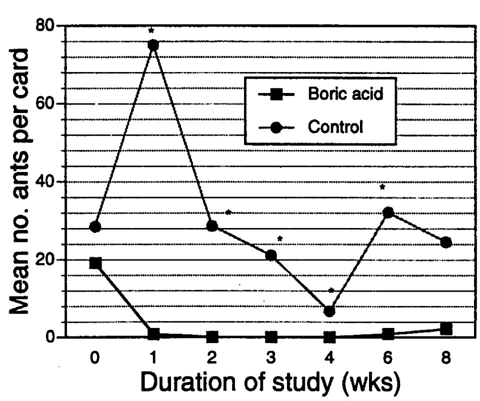

Fig. 5. Mean number of *Monomorium pharaonis* per card obtained inside and outside apartment buildings, one to eight wk following placement of bait stations. Means with an asterisk are significantly different (P<0.05) from the control using GLM on $\log_{10}(x+1)$ transformed data. Untransformed means are shown in graph.

with bait and enclosed in a station with small holes to allow ant entry and prevent evaporation. And finally, addition of preservatives to extend the shelf-life of the bait will be necessary. Each of these factors must be tested and evaluated in order to develop a bait which will ultimately be successful.

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