FACTORS AFFECTING the PERFORMANCE of BAIT TOXICANTS for ARGENTINE ANTS (HYMENOPTERA: FORMICIDAE)

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Abstract Argentine ants, *Linepithema humile* (Mayr), are a major urban pest species in many countries with Mediterranean and semi-tropical climates. *L. humile* primarily feeds on honeydew, nectar, and other liquid sweets. Baits consisting of sucrose solutions are highly preferred. Sucrose-based liquid solutions containing imidacloprid exhibited delayed toxicity, were non-repellent, and provided good reductions of ants foraging around structures. Optimal concentrations ranged from 0.0005 to 0.005%, a 100-fold range in effective rate. Boric acid in sucrose water did not exhibit delayed toxicity. Only 0.5% to 1.0% boric acid provided slow toxicity to *L. humile*, the optimal range being just 2-fold. A commercial bait containing 1.0% boric acid provided statistically significant reductions, but the level of control was unacceptable. Reduced attractiveness and evaporation of water from the baits probably contributed to the reduced performance in the field. A wide range of effective concentrations is therefore desirable under field conditions.

Key Words *Linepithema humile*, pest management, control, imidacloprid, boric acid

INTRODUCTION

Baits have been recommended for the control of Argentine ants, *Linepithema humile* (Mayr), for nearly a century (Rust, 1986); however, we have not tested any commercial bait that provides consistently good control. The performance of baits for Argentine ants is affected by biotic factors such as the developmental stage and nutritional requirements of the colony and the repellency or avoidance of toxicants by workers, and abiotic factors such as the type of toxicant, formulation of the bait, and the bait delivery system. Stringer et al. (1964) proposed several criteria important for baits to be effective against red imported fire ants, *Solenopsis invicta* Buren: "(1) exhibit delayed action over at least a 10- to 100-fold dosage range and preferably greater, (2) be readily transferred between ants and kill the recipients, and (3) not be repellent when combined with a bait."

In baits for *L. humile*, concentrations of boric acid > 1% in 25% sucrose water significantly decreased consumption (Klotz et al., 2000). Hooper-Bui and Rust (2000) found that a 24-h exposure to fipronil baits in sucrose water provided excellent worker kill at concentrations of 1 x 10-5 to 1 x 10-3 % and 100% queen kill at 1 x 10-5 and 1 x 10-4 %. Higher concentrations killed workers too fast. Continuous exposure of 0.5% boric acid in sucrose water provided excellent kill of workers and queens whereas abamectin and hydramethylnon sucrose baits killed workers, but not queens. Certain toxicants such as chlorpyrifos and hydramethylnon readily killed workers but did not provide delayed toxicity necessary to kill the queens (Knight and Rust, 1991). Hooper-Bui and Rust (2001) demonstrated the importance of slow-acting toxicants in a study where about 90% of the workers were killed when they ingested 1.03 ìg of hydramethylnon per mg of ant body weight whereas only 60% of the queens were killed. With toxicants such as hydramethylnon multiple feedings of queens are necessary to kill them.

Proceedings of the 4th International Conference on Urban Pests.
Susan C. Jones, Jing Zhai, and Wm H. Robinson editors. (2002)

The objectives of this study were to determine the optimal concentrations of boric acid and imidacloprid in sucrose–water baits against Argentine ants. We report LT50 toxicity studies and bait acceptance of boric acid and imidacloprid baits and examines their relationship to field efficacy.

MATERIALS and METHODS

Toxicity Tests

To determine the time required to kill *L. humile*, workers were provided toxic bait solutions in small petri dishes (Klotz et al., 2000). The ants were provided water but no food for 24 h prior to the tests. Crystalline boric acid (99% [AI]; Sigma, St. Louis, MO) was dissolved in 25% sucrose-deionized water solutions to provide concentrations ranging from 0.125% to 5%. Imidacloprid (98.7% tech., Bayer Corp., Kansas City, MO) was initially dissolved in 3 ml of acetone and added to 100 ml of 25% sucrose-deionized water solutions to provide concentrations ranging from 0.0001% to 0.01%. Bait solutions were added to cotton plugs daily in side small petri dishes (2 cm by 5 cm diam) with 10 ants. Treatments and controls (25% sucrose water) were replicated 10 times. Daily observations on cumulative mortality were recorded for 7 d.

The time required to produce 50% kill of workers was determined by linear interpolation for each replicate. The average (± SEM) was determined for the 10 replicates.

Acceptance Studies

Bait acceptance studies were conducted in feeding arenas that provided the ants with a range of solutions to choose. The arenas were made from aluminum cake pans (20 cm diam) with four holes punched through the sides above the bottom of the pan approximately 90º apart. Sections of glass tubing (10 cm) were inserted in the holes so that their ends were flush with the edge of the pan and the other ends directed towards the center of the pan. Ten plastic weigh boats were cemented to the floor of the cake pan to hold 1.5-ml centrifuge tubes that contain the bait solutions. The tubes were filled with solution and weighed (about 1.5 g). Solutions consisted of serial dilutions of each toxicant in 25% sucrose water along with a control. The arenas were covered with a piece of plywood to prevent animals from disturbing the solutions and placed near *L. humile* nests. The arenas were periodically examined and then removed when one of the bait solutions was nearly empty. The arenas were returned to the lab and the tubes and solutions weighed. A minimum of nine replicates was tested for each toxicant.

Field Tests

Bait efficacy around homes in Riverside, California was calculated from a reduction in ant foraging, based on adjusted weight loss from monitoring tubes of sucrose water before and after treatment rather than based on numbers of ants counted or trapped. Twenty 15-ml polypropylene tubes (Falcon screwcap vials) filled from a 1-qt capacity repeating pipette (Model 9020 Repipette II, Barnstead International, Dubuque, IA) with about 13 ml of 50% (wt/wt) sucrose water were placed around each home for about 24 hours. Based on a laboratory study, foraging *L. humile* imbibe an average of 0.3 mg sucrose water per visit, essentially doubling their weight. The tubes were placed no closer that about 7 m from one another, adjacent to the structure and around the perimeter of the yard. The tubes were laid on their side, with the mouth of the vial supported on a small wooden pedestal to prevent the liquid from spilling. Loss or gain of liquid (i.e., weight) from the tubes was corrected for evaporation and drowned ants. The adjusted weight loss value made it possible to estimate the number of ant visits per station, and to map areas of greatest foraging. There is a direct correlation between amount of sugar water taken, ant visits, and the number of ants in the area. In other words, lower numbers of visits indicates lower overall ant numbers. One advantage of this monitoring technique is that it reflects long-term foraging (i.e. 24 hr) and does not depend on a singular momentary observation that may vary greatly with time of day. Only residences in which there was initially significant ant feeding at 8 or more stations were used in the study. At least 6 ml taken was considered significant.

Imidacloprid baits were placed in the field into clear plastic tubes (23 cm long by 4.2 cm diameter) in which three small holes (1.1cm) were drilled in the tube (Klotz et al., 1998). Pieces of evaporative Coolpad (Research Products, Phoenix, AZ) were cut and placed inside the tubes and the tubes were capped. The aqueous baits were poured into the tubes and then placed inside slightly large pieces of white PVC pipe (25.5 cm long by 6 cm diam). The outer piece of PVC pipe protected the baits from animals and reduced evaporation of the baits. Ten bait stations were placed around the perimeter of each yard, no closer than 3 m to the structure. The baits were removed at day 6, 13, 20, and 27 and returned to the lab so that they would not interfere with the monitoring stations. Fresh bait was poured in to each station and the baits were returned to the field on day 7.

Boric acid bait (1.0%, Drax Liquidator, Waterbury Co. Inc., Waterbury, CT) was placed in a plastic commercial bait station (Waterbury Co. Inc., Waterbury, CT).

Data Analysis

In the choice studies, bait consumption was analyzed with a Friedman's test or a two-way analysis of variance by ranks (Conover, 1971). In field studies, the number of ants visits before and after baiting was analyzed with a Wilcoxon signed-ranks test (Sokal and Rohlf, 1969).

RESULTS

Concentrations of 0.0005% to 0.005% imidacloprid provided LT50s ranging from 3.6 to 0.78 days (Figure 1). Higher concentrations provided an LT50 of about 0.5 days and killed workers too quickly. Concentrations lower than 0.0005% failed to provide 50% kill within 7 days. The low amount of bait consumed at 0.0005% is probably an anomaly because there was more consumption at both the higher and lower concentrations. It is not uncommon in choice tests to have an occasional low feeding point when 4 or 5 different concentrations of bait and the 25% sucrose control are heavily fed upon. When several highly preferred food sources are available to the ants, foraging trails are quickly established to them. With 7- to 24-hour exposure times minor differences in acceptance are not revealed. Although there were no significant differences in feeding among the different concentrations, the 0.05% imidacloprid ranked 7th in 3 of the 6 trials.

In choice tests with boric acid and sucrose water, the amount of feeding varied greatly among all concentrations including the 25% sucrose water control (Figure 2). There was no significant difference in consumption among the various concentrations over 7 h. However, the 5% boric acid ranked 6th or 7th in 5 of the 9 trials.

The imidacloprid sucrose-water bait provided statistically significant reductions in the number of ants foraging at the monitors (Table 1). The 0.001% imidacloprid bait provided consistently greater reductions in ant counts that did the 0.0025% or 0.005% baits. Initially, ants foraged all three concentrations, but after 7 days considerably more of the 0.001% imidacloprid bait was consumed. Many of the stations were empty at 7 days and additional bait was added. The 1.0% boric acid bait provided statistically significant reductions in ant counts at each post-treatment period compared with the pre-treatment count. However, it failed to provide acceptable reductions in the number of ants surrounding the structures.

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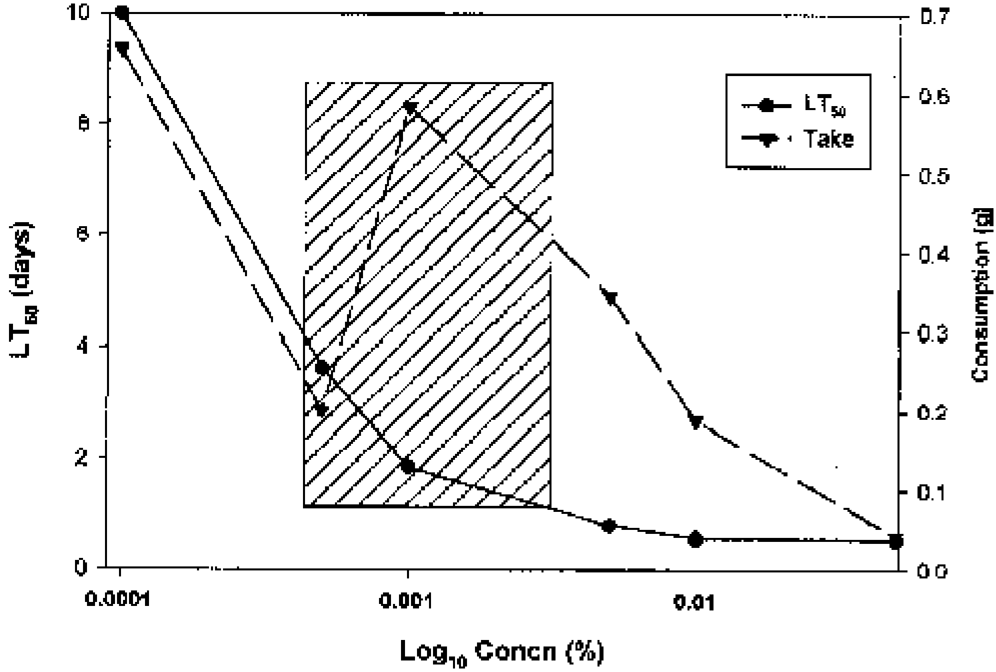

Figure 1. The time required for various concentrations of imdiacloprid + 25 sucrose water to kill worker L. humile *and the amount of bait consumed in choice tests. The rectangle denotes the concentrations that provide optimal kill and acceptance.*

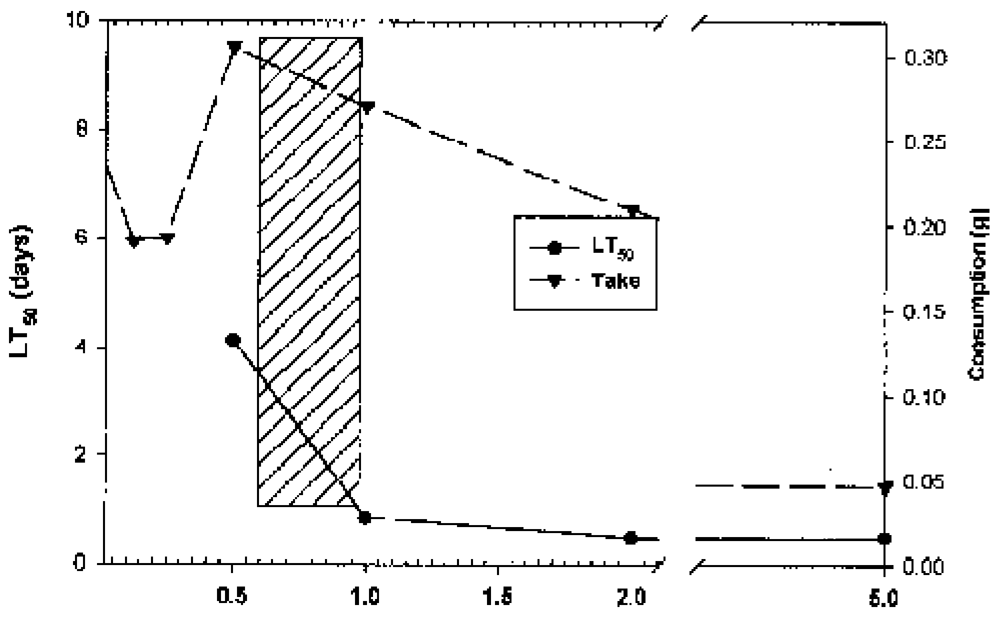

Figure 2. The time required for various concentrations of boric acid + 25 sucrose water to kill worker L. humile *and the amount of bait consumed in choice tests. The rectangle denotes the concentrations that provide optimal kill and acceptance.*

Table 1. Efficacy of 25% sucrose-water baits against Argentine ants, *Linepithema humile*

Bait	Avg. Precount Ant visits/ vial	Avg. No. ant visits (% reduction)[a]			
		1 wk	2 wk	3 wk	4 wk
Imidacloprid, 0.005%	26,918	16,272 (39.5)*	18,222 (32.3)*	20,470 (24.0)*	17,911 (33.5)*
Imidacloprid, 0.0025%	38,788	19,815 (48.9)*	19,936 (48.6)*	12,418 (68.0)*	21,128 (45.5)*
Imidacloprid, 0.001%	29,779	10,628 (64.3)*	9,812 (67.1)*	9,003 (69.8)*	11,082 (62.8)*
Boric acid[b], 1.0%	32,639	20,799 (36.3)*	21,183 (35.1)*	28,219 (13.5)*	22,819 (30.0)*

[a] Means followed by asterisk are significantly different at $P<0.05$ (Wilcoxon signed-ranks test).
[b] Drax Liquidator 1.0% boric acid. (Waterbury Co. Inc, Waterbury, CT). Baits applied 7/5/2001.

DISCUSSION

Baits must contain toxicants that have delayed action over at least a 10-fold range of concentrations, are not repellent, and readily transferred by trophallaxis (Stringer et al., 1964). Baits for red imported fire ants incorporate toxicants dissolved in soybean oil and applied to corn grit. Baits such as Amdro® are generally unacceptable to many species of Dolichoderine ants that feed on honeydew such as *L. humile*; pyramid ants, *Conomyrma insana* (Buckley) and *C. bicolor* (Wheeler); velvety tree ant, *Liometopum occidentale* Emery; and odorous house ant, *Tapinoma sessile* (Say) (Wagner, 1983). In developing ant baits for sweet-feeding ants, several other factors besides those described above must also be considered. If aqueous sugar solutions are used, then the toxicants must have some solubility in water at least to the minimum lethal dose required to kill workers or they must be formulated in such a manner to insure their stability in aqueous bait. For example, 25 % sucrose solutions of 5 x 10-4% fipronil bait were preferred over similar fipronil concentrations in a sucrose gel matrix (Silverman and Roulston, 2001). Secondly, evaporation of the water is extremely important because it increase the concentration of the toxicant. Some commercial stations lose up to 26% of the water in just 7 days, and more than 60% in 14 days (Reierson and Rust, unpublished data).

Imidacloprid bait, 0.001% meets the criteria established by Stringer et al. (1964). The concentrations for delayed toxicity (LT50 between 1-3 days) range between 10 to 25-fold. It also meets our standard because imidacloprid has sufficient water solubility to permit mixing with 25% sucrose water. The field data confirms that concentrations of about 0.001% are effective in reducing the number of foraging ants. Slightly lower concentrations (0.0005%) will also be effective and while simultaneously providing some additional guard against evaporation.

Boric acid in 25% sucrose does not meet the necessary criteria of a delayed action bait. Only 1.0 and 0.5% boric acid produces an LT50 between 1 and 3 days. Higher concentrations provided significantly faster mortality. Drax Liquidator provided a LT50 of 1.9 ± 1.02 h. Hooper-Bui and Rust (2000) found that only 0.5% boric acid in sucrose water baits killed all workers and queens within 14 days. Knight and Rust (1991) reported that a commercial 5% boric acid gel was not readily accepted by workers and failed to kill queens. In paired choice tests with 25% sucrose water, Klotz et al. (2000) found that consumption significantly decreased when baits contained 2 or 4% boric acid. Consumption was reduced by 80-90% compared with 0.5 and 1.0% boric acid sucrose baits. In choice tests, Drax Liquidator and several other commercial boric acid baits were not well accepted suggesting that other additives in these formulations might be repellent (Rust et al., unpublished data). Our data suggests that lack of performance of = 1.0% boric acid in sucrose water is attributed to their quick kill and repellency. Fast-acting toxicants prevent the workers from establishing recruitment trails. Caution must be exercised when comparing data generated with technical boric acid in sucrose solutions and commercial baits.

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Field tests with a commercial 1.0% boric acid bait provided significant reductions in the number of ants foraging at monitoring stations. However, as expected, the decreased acceptance and the fast action of the 1.0% bait prevented ants from long-lasting trails to the baits. Consequently, the reductions never exceeded 36%. Evaporation of water was a problem in the bait stations and the baits became more concentrated over 4 wk. Klotz et al. (1998) found that 0.5% boric acid in 25% sucrose-water bait provided a significant and continuous reduction of L. humile. They visually estimated a 80% reduction in the number of ants visiting the bait stations. All our data suggest that 0.5% boric acid provides better control than do higher concentrations and allows for some evaporation.

Several factors influence the effectiveness of potential toxicants in baits for Argentine ants. The toxicant should provide delayed toxicity over at least a 10-fold concentration range. It must not be repellent and readily transferred among workers. Lastly, it should have sufficient water solubility to insure mixing into sucrose solutions. The design of the bait station from which the bait is dispensed is extremely important because the large volume stations are needed and evaporation of water can concentrate the bait. The potential for developing a liquid bait system for Argentine ant control looks very promising.

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