

# Laboratory Evaluation of Boric Acid-Sugar Solutions as Baits for Management of German Cockroach Infestations

J. CHAD GORE AND COBY SCHAL<sup>1</sup>

Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613

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**ABSTRACT** Boric acid dust has a long history as an insecticide in urban pest management, and it has been shown to be an effective alternative to conventional neurotoxic insecticides. However, dust formulations require specialized equipment and are difficult to apply, whereas gel and paste formulations contain large amounts of boric acid and tend to be less efficacious than other insecticide baits. The purpose of this study was to evaluate the effectiveness of borate solutions as baits against the German cockroach. Several borate-sugar combinations were evaluated in choice and no-choice assays in the laboratory. Mortality was recorded for 15 d and expressed as lethal time<sub>90</sub>, the time taken to kill 90% of the cockroaches. Results showed that boric acid was more effective than sodium tetraborate or disodium octaborate tetrahydrate and that aqueous solutions containing mixtures of 0.5–2% boric acid and any of several inexpensive sugars, including fructose, glucose, maltose, and sucrose as a phagostimulant, at molar concentrations of 0.05–1.0, can provide rapid and effective kill of German cockroaches, *Blattella germanica* (L.).

**KEY WORDS** German cockroach, boric acid, liquid bait, insecticide, IPM

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BORIC ACID ( $H_3BO_3$ ) dust, a nonvolatile, slow-acting inorganic insecticide, has long been used in urban pest management (Ebeling 1995). Negligible absorption of boric acid through unbroken skin and relatively low mammalian toxicity (Fail et al. 1998, Hubbard 1998, Wester et al. 1998) have contributed to its favorable safety record as an insecticide. Nevertheless, after World War II, the use of inorganic insecticides in structural pest management gave way to organic, neurotoxic compounds that provided much faster kill. For almost 40 yr, conventional cockroach management has thus shifted to greater reliance on spray applications of residual formulations of broad-spectrum organic insecticides, primarily organophosphates and pyrethroids. In the last two decades, however, insecticide baits have largely displaced other formulations for control of German cockroaches and other structural pests, especially as components of integrated pest management (IPM) programs (Appel 1990, Reiersen 1995). Because insecticides in baits are part of a solid or gel matrix that is applied in cracks and crevices, the toxins are typically much less translocatable and therefore less hazardous than in spray formulations. However, baits containing 30–50% boric acid have been only marginally effective against the German cockroach (Appel 1992).

Recent regulatory restrictions imposed by the U.S. Food Quality Protection Act of 1996, the large financial obligation required for pesticide registration,

health and environmental concerns, and the emergence of insecticide resistance in urban pests have led to a vigorous search for alternative, cost-effective pest management approaches, including a reexamination of boric acid dust (Zurek et al. 2002, 2003). However, dust formulations are usually more difficult to apply, require specialized equipment, and can result in drift and translocation in windy conditions. Therefore, alternative formulations have been sought to counter these limitations.

A favorable property of boric acid is its relatively high solubility in water and apparent lack of repellency to German cockroaches, *Blattella germanica* (L.) (Ebeling et al. 1966, Strong et al. 1993). Water can thus serve as a matrix into which water-soluble attractants such as aggregation pheromones, food odorants, and phagostimulants (Tsuji 1965, Sugawara et al. 1975, Rust and Reiersen 1977, Wiley and Boush 1983) may be added. Although recent studies have shown that liquid baits containing boric acid and sucrose can be effective against several ant species (Klotz and Moss 1996; Klotz et al. 1996, 1997a,b) and house flies (Hogsette and Koehler 1994), such formulations have not been tested against cockroaches. The goal of our study was to develop liquid borate baits for the control of German cockroach populations. We undertook four objectives, namely, to 1) determine which of three borates would be most effective, 2) determine which of various sugars is most effective as a phagostimulant in a borate solution, 3) develop dose-mortality curves with various borate-sugar combina-

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<sup>1</sup> E-mail: coby\_schal@ncsu.edu.

tions, and 4) evaluate the efficacy of a prototype aqueous bait under field conditions. Herein, we report the results of the first three aims, whereas the field evaluation is reported separately (Gore et al. 2004).

### Materials and Methods

**Insects and Experimental Cages.** Adult males (12–18 d old) and females (1–2 d old) were collected from a synchronously reared laboratory colony of insecticide-susceptible German cockroaches (American Cyanamid strain, Princeton, NJ). Cockroaches were supplied ad libitum with rat chow (Purina No. 5012, Purina Mills, St. Louis, MO), and water was provided in glass tubes with cotton stoppers. The inner vertical surfaces of Plexiglas cages (small cage, 30 by 15 cm; large cage, 30 by 117 cm) were coated with a thin layer of petroleum jelly to prevent cockroaches from escaping. Each cage was provided a paper egg carton for refuge. Cockroaches were allowed to acclimate in cages for 48 h before the start of each assay.

**Borate Assays.** In no-choice assays, 20 adult females were provided water vials containing various concentrations (wt:vol) of boric acid (Fisher, Fair Lawn, NJ), sodium tetraborate (borax) (Sigma, St. Louis, MO), or disodium octaborate tetrahydrate (DOT) (MOP-UP, Waterbury Companies, Independence, LA) without access to clean water. In two-choice assays, females were provided a choice of distilled water and one of the three borates. Each assay was conducted twice in both small and large cages. Mortality was recorded daily for 15 d.

**Sugar Assays.** Choice assays consisted of 24 h, 48 h, or continuous exposures to water vials containing 0.5 or 1% boric acid with and without 0.1 M sugar in either small ( $n = 20$  females) or large ( $n = 50$  males) cages. In a preliminary screen of sugars, assays were conducted only once after exposure to baits for 24 or 48 h, but assays of continuous exposure were replicated ( $n = 40$  females). Sugars assayed were D(-)-fructose (ICN Biochemicals, Cleveland, OH), D-(+)-sucrose (Fisher), maltotriose, D-(+)-melezitose, D-(+)-trehalose, D-(+)-maltose, palatinose, L(-)-sorbitol, D(-)-mannose, D-(+)-glucose, D-(+)-galactose, D-(+)-xylose, D(-)-ribose, L-(+)-arabinose, and D(-)-arabinose (Sigma). The glycoside *p*-nitrophenyl  $\alpha$ -D-glucopyranoside (Sigma) was also assayed. Mortality was recorded daily for 15 d.

**Combined Boric Acid and Sugar Assays.** In each assay, 30 males in a small cage were given a 48-h choice between distilled water and a boric acid-sugar solution. Sugars were selected based upon the preliminary screen described above, and they included maltose, sucrose, glucose, and fructose at concentrations ranging from 0.01 to 2.0 M. Boric acid was assayed at concentrations of 0.5, 1.0, and 2.0% (wt:vol). Controls consisted of water vials that contained boric acid but no sugar. Mortality was recorded daily for 15 d.

**Data Analysis.** Lethal time ( $LT_{90}$ ) values were generated by PROC UNIVARIATE and analyzed by analysis of variance (ANOVA) (PROC GLM) in SAS 8.2 (SAS Institute 2001) to determine whether borate

type, sugar type, combinations of boric acid and sugars, and their concentrations affected mortality. Treatment means were compared using Fisher's least significant difference (LSD) ( $\alpha = 0.05$ ).

### Results

**Comparison of Borates.** Days to 90% mortality ( $LT_{90}$  values) were determined in response to three different borates in no-choice and choice assays in small cages. All three borates resulted in relatively rapid mortality when females were confined in small cages and forced to drink 0.5–5% borate-laced water (no-choice assays) (Fig. 1A; Table 1); none of the control females exposed to clean water without borates died in these assays. At all concentrations  $>2\%$ , boric acid killed cockroaches faster than the other borates ( $F_{1, 71} = 480$ ;  $P < 0.0001$ ;  $t_6 = 2.45$ ;  $P < 0.05$ ). This pattern was even more pronounced in two-choice assays (Fig. 1B; Table 1). Mortality was significantly slower ( $t_{36} = 2.03$ ,  $P < 0.05$ ) in two-choice assays than in no-choice assays. Furthermore, given a choice of clean water and various concentrations of borax- or DOT-water, females avoided the borates and survived the 15-d assays. However, females exposed to boric acid under these conditions exhibited a significant dose-dependent mortality, indicating that there was minimal avoidance of this insecticide (Fig. 1B).

Because the two choices were only 12.5 cm from each other in the small cages, females could easily sample both choices while foraging. Larger cages represent more realistic foraging conditions, and we expected females to less clearly discriminate among the borates. In fact, we obtained similar results using large cages (Fig. 1C and D; Table 1). Under these conditions females also avoided disodium octaborate tetrahydrate and sodium tetraborate in favor of clean water, but they did not avoid the boric acid bait. Based on these results, only boric acid was included in further studies.

**Comparison of Sugars.** To compare the palatability of various sugars, we exposed 50 males for 24 or 48 h to a choice of clean water and baits containing 0.1 M sugar and 1% boric acid in water; survivorship was monitored for 15 d. Only two sugars, maltose and sucrose, caused males to consume lethal amounts of boric acid during the 24-h exposure (Fig. 2). Most other sugars resulted in lower  $LT_{90}$  values after a 48-h exposure in large cages, but none performed significantly better than maltose and sucrose. Boric acid solutions containing ribose or sorbitol failed to kill 90% of the cockroaches within 15 d.

These results were confirmed with 1–2-d-old females (feeding stage) continuously exposed in small cages to a choice of clean water and water solutions containing 0.5% boric acid and 0.1 M sugar. Although many sugars were equally effective in stimulating females to consume boric acid, several, including ribose and sorbitol, were weakly active (Fig. 3). The glycoside *p*-nitrophenyl  $\alpha$ -D-glucopyranoside was the least effective in these assays. Based upon the results of these screens of various carbohydrates, and consider-

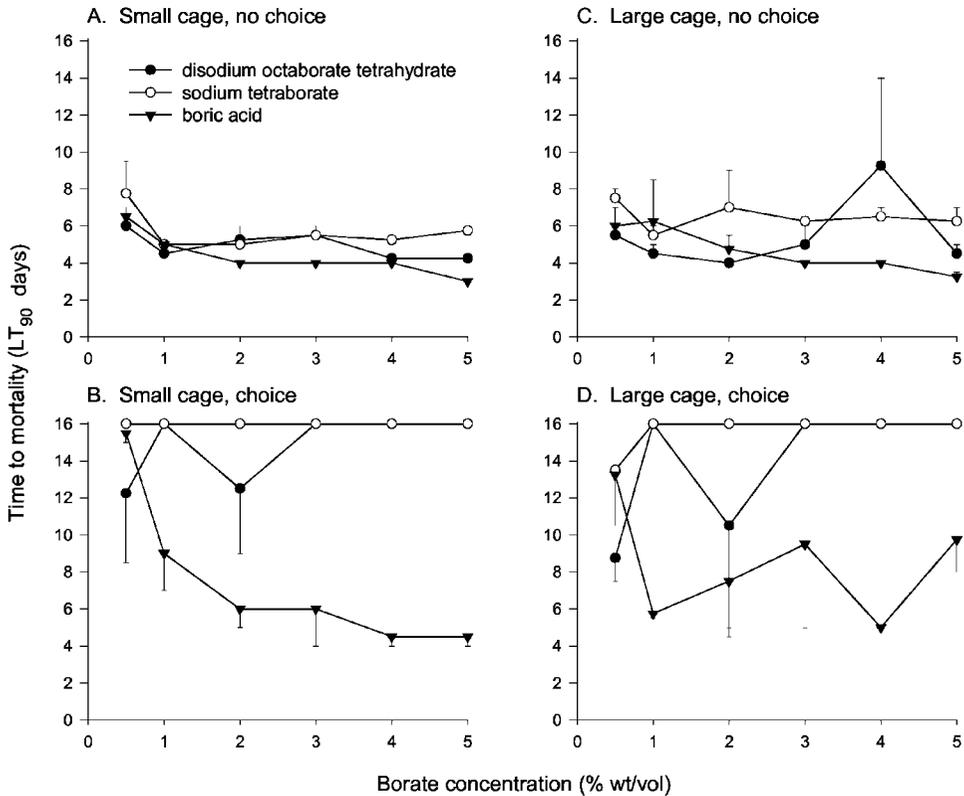


Fig. 1. No-choice and choice mortality assays with water solutions of various concentrations of three different borates in small and large cages. Clean water was also provided in the choice assays.  $LT_{90}$  values represent days to 90% mortality from univariate analyses. Each point represents the average ( $\pm$ SEM) of duplicate assays, each with 20 adult females.

ing their cost for future implementation in pest management programs, we selected maltose, sucrose, glucose, and fructose for further assays.

Complete survival was observed in control cohorts of male cockroaches that were not exposed to boric acid (data not shown). Solutions of 0.5, 1.0, and 2.0% boric acid without sugar caused dose-dependent mortality with  $LT_{90} = 5.8$  d at 2% boric acid and  $LT_{90}$  values exceeding 15 d at 0.5% boric acid (Fig. 4).

Table 1. Time (days) to 90% mortality ( $LT_{90}$ ) for all concentrations combined of each borate in no-choice and choice assays in small and large cages

Assay	Borate <sup>b</sup>	N	$LT_{90} \pm$ SEM (d) <sup>a</sup>	
			Small cages	Large cages
No-choice	Borax	12	5.7 $\pm$ 0.43a	6.5 $\pm$ 0.28a
No-choice	DOT	12	5.0 $\pm$ 0.30b	5.5 $\pm$ 0.79a,b
No-choice	Boric acid	12	4.4 $\pm$ 0.49b	4.7 $\pm$ 0.49b
Choice	Borax	12	16.0 $\pm$ 0.00a	15.6 $\pm$ 0.42a
Choice	DOT	12	14.8 $\pm$ 0.76a	13.9 $\pm$ 1.36a
Choice	Boric acid	12	7.6 $\pm$ 1.72b	8.5 $\pm$ 1.24b

$LT_{90}$  values represent time to 90% mortality. Values  $>15$  d represent  $<90\%$  mortality at the conclusion of the 15-d bioassay.

<sup>a</sup> Means within columns and within assay type (choice, no-choice) with the same letter are not significantly different by using LSD.

<sup>b</sup> Borax is sodium tetraborate, and DOT is disodium octaborate tetrahydrate.

The concentration of boric acid and the concentration of sugar significantly affected mortality of cockroaches ( $F_{4, 155} = 77.49$ ;  $P < 0.0001$  and  $F_{5, 155} = 56.34$ ;  $P < 0.0001$ , respectively; Fig. 4). Although ANOVA indicated that the type of carbohydrate alone did not affect mortality, a significant interaction of sugar concentration and boric acid concentration ( $P = 0.0007$ ) indicated that different sugars resulted in significantly different levels of mortality at different dose combinations. At low boric acid levels (0.5 and 1%), all four sugars were most effective at intermediate molar concentrations, and less so at low and high sugar concentrations. At 2% boric acid, the addition of sugar contributed less to the  $LT_{90}$  values, but at concentrations of sugar  $>1$  M the baits were aversive to cockroaches and thus resulted in delayed mortality (Fig. 4).

Mixtures of glucose and boric acid resulted in dose-dependent mortality ( $F_{17, 35} = 5.43$ ;  $P = 0.0004$ ; Fig. 4A). Whereas no mortality was observed with 0.5% boric acid alone, the addition of 0.1, 0.5, and 1.0 M glucose resulted in significant mortality within 15 d. With 1% boric acid,  $LT_{90}$  values were lowest (rapid mortality) when 0.1 or 0.5 M glucose was added. However, higher molar concentrations of glucose were aversive to cockroaches and 2.0 M glucose significantly delayed mortality relative to 1% boric acid baits

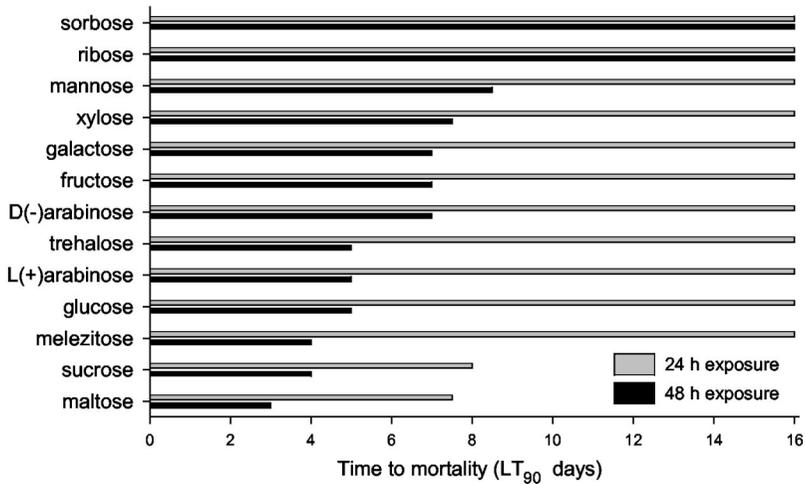


Fig. 2. Time to death ( $LT_{90}$  in days) after 24- or 48-h exposure to solutions of 0.1 M sugar and 1.0% boric acid in large cage choice assays. Fifty adult males were monitored for each exposure time-sugar combination.  $LT_{90}$  values  $>15$  d represent  $<90\%$  mortality at the conclusion of the 15 d assay. See *Materials and Methods* for complete designations of sugar stereoisomers.

without glucose ( $15.5 \pm 0.5$  versus  $10.8 \pm 1.9$ ;  $t_{81} = 1.99$ ,  $P < 0.05$ ; Fig. 4A). Addition of glucose to a 2% boric acid solution failed to significantly hasten mortality. Nevertheless,  $LT_{90}$  values as low as 3.0 d were obtained with mixtures of 2% boric acid and 0.05 M glucose.

Baits with the monosaccharide fructose resulted in similar dose-dependent mortality to glucose ( $P < 0.0001$ ; Fig. 4B). However, unlike glucose, cockroach mortality on 1% boric acid and low fructose concentrations (0.01 and 0.05 M) was significantly greater than without fructose.

The disaccharides sucrose and maltose were more effective than the monosaccharides at equivalent molar concentrations. At 0.5 and 1% boric acid,  $LT_{90}$  values were lowest with 0.05–0.5 M sucrose solutions

and increased at both lower and higher concentrations of sucrose ( $P < 0.0001$ ; Fig. 4C). With maltose, 0.5 and 1% boric acid solutions killed cockroaches even more rapidly than sucrose at equivalent concentrations of 0.01 M (Fig. 4D).

## Discussion

Borates have been used primarily as dusts for management of structural pests. Dust formulations take advantage of the grooming habits of insects, whereby insecticide is ingested after being removed from antennae and other appendages. However, repellency of some dusts has seriously limited their efficacy on cockroaches. Borax dust, for example, is moderately repellent to cockroaches and adheres less to their cuticle

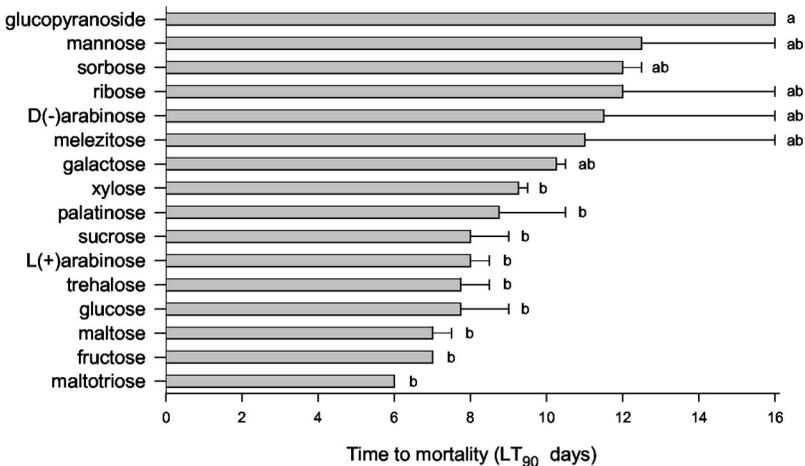


Fig. 3. Time to death ( $LT_{90}$  in days) under continuous exposure to solutions of 0.5% boric acid and 0.1 M sugar. Values expressed as  $LT_{90} \pm$  SEM in days and  $LT_{90}$  values  $>15$  d represent  $<90\%$  mortality at conclusion of the 15-d assay. Each bar represents the average ( $\pm$ SEM) of duplicate assays, each with 20 adult females. See *Materials and Methods* for complete designations of sugar stereoisomers.

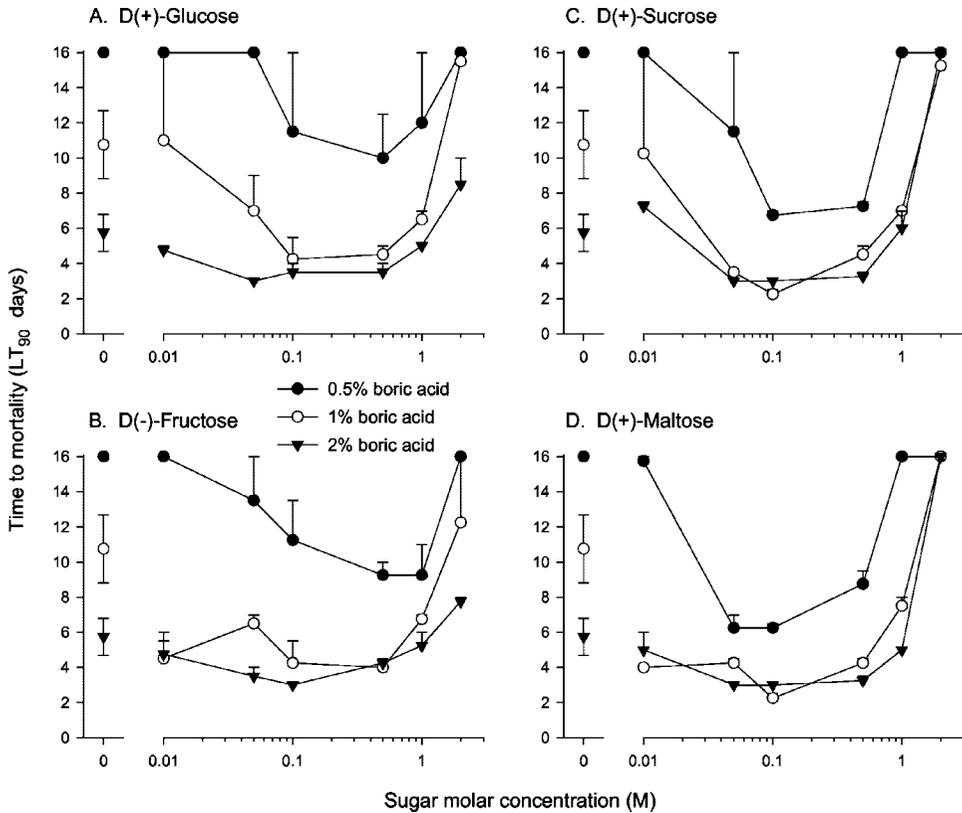


Fig. 4. Dose-mortality curves for boric acid-sugar combinations in water, reflecting preference-aversion decisions by cockroaches. Adult males ( $n = 30$ ) in a small cage were exposed for 48-h to a choice of clean water and water containing boric acid and sugar. Mortality is expressed as the mean  $LT_{90}$  values (in days) of two replicates  $\pm$  SEM.  $LT_{90}$  values  $>15$  d represent  $<90\%$  mortality at the conclusion of the 15-d assay.

than does boric acid (Ebeling et al. 1966). Unlike other inorganic insecticides and many fast-acting organic insecticides, boric acid is not repellent to German cockroaches when properly used as either a dust (Ebeling et al. 1966) or as bait (Strong et al. 1993). Because boric acid has a favorable safety record and no known cases of resistance in insects, it was extensively used before the advent of fast-acting organic insecticides, including carbamates, organophosphates, and pyrethroids. Although it has been suggested that cuticular penetration and the destruction of the foregut epithelium contribute to its insecticidal activity (Cochran 1995, Ebeling 1995), the mode of action of boric acid remains unresolved. Sublethal effects, including premature drop of oothecae and reduced hatching success, may also contribute to its efficacy (Barson 1982, Zhou and le Patourel 1990).

Our recent research has shown that boric acid dust is effective as a stand-alone treatment for cockroach infestations in swine farms (Zurek et al. 2003) or in combination with *Metarhizium anisopliae* in laboratory assays (Zurek et al. 2002). However, extensive dust applications, as required in agricultural settings, entail specialized and expensive equipment. Also, such applications, including in residential settings, may expose occupants and applicators to respiratory

health risks associated with dust inhalation. Therefore, our goal has been to develop and evaluate aqueous solutions of borates for cockroach control.

Our first step in the development of aqueous borate baits was to determine which borate would be most effective. Although disodium octaborate tetrahydrate solutions are significantly more toxic to cockroaches than boric acid solutions in no-choice tests, Strong et al. (1993) reported that disodium octaborate tetrahydrate was also more repellent than boric acid. Our results showed that solutions of disodium octaborate tetrahydrate and boric acid performed equally but significantly better than borax in no-choice assays (Table 1). In choice assays, however, boric acid consistently performed better than either disodium octaborate tetrahydrate or borax, suggesting that cockroaches are less averse to eating boric acid. We therefore excluded disodium octaborate tetrahydrate and borax from further considerations in bait development. Because the cotton surface became encrusted with crystallized boric acid at high ( $>2\%$ ) concentrations, we decided to consider more dilute boric acid solutions in subsequent assays.

Second, we evaluated whether various sugars could enhance the insecticidal activity of boric acid solutions. It is important to note that water itself consti-

tutes a vital and attractive resource for cockroaches. Cockroaches drink regularly (Cochran 1983) and survive longer in higher than in lower humidity (Dambach and Goehlen 1999). Numerous reports have suggested that reducing water availability, for example, through improved sanitation, can reduce cockroach infestations (Schal and Hamilton 1990, Appel 1997). The trend in the last decade to deploy gel baits in place of solid insecticide baits or pastes also is largely based upon greater acceptance by cockroaches of bait formulations with high water content. Other urban pests, Argentine ants, for example, consume twice as much liquid sucrose bait as gel formulations of the same bait (Silverman and Roulston 2001). Because boric acid seems to interfere with water balance, insects may also elevate ingestion rates of boric acid solutions to counteract dehydration (Klotz et al. 1996, Strong et al. 1993).

Our ultimate goal is to control cockroach populations with a boric acid-sugar bait. We therefore monitored cockroach mortality on sugar-boric acid mixtures as an indirect measure of bait intake, rather than directly measure ingestion of various sugars. For this reason, we also measured  $LT_{90}$  rather than  $LT_{50}$  values. Several carbohydrates were clearly ineffective in these assays, including some highly nutritious sugars, such as galactose and mannose, as well as sugars with relatively less nutritional value, such as ribose and sorbose (Figs. 2 and 3). We included *p*-nitrophenyl  $\alpha$ -D-glucopyranoside in these assays because in the blowfly *Phormia terraenovae* Robineau-Desvoidy it was  $\approx 10$ -fold more effective than sucrose at stimulating labelar electrophysiological responses (Dethier 1976). However, this glycoside was least effective in our assays with the German cockroach (Fig. 3).

Tsuji (1965) found that L-arabinose, maltose, sucrose, glucose, and fructose were moderately to highly effective feeding stimulants to cockroaches. Our results generally corroborate Tsuji's findings. Water-based baits containing 0.5 or 1.0% boric acid killed cockroaches significantly faster when fortified with the monosaccharides fructose, glucose, and L-arabinose, the disaccharides maltose, sucrose, and trehalose, or the trisaccharides maltotriose and melezitose (Figs. 2 and 3). Nevertheless, several of these sugars, for example, melezitose, are prohibitively expensive for practical pest control. Arabinose, however, may be of interest for future investigations because it is relatively non-nutritious (Dethier 1976), yet it stimulates feeding, a combination that might result in greater long-term ingestion.

Our evaluation of the four most active sugars in combination with boric acid revealed that maximum mortality was typically achieved at low-to-moderate sugar molar concentrations (Fig. 4). With 1% boric acid and sugar concentrations  $<0.1$  or  $>1$  M, these mixtures became increasingly less effective; little or no mortality was observed at 2.0 M sugar. Although behavioral aversion of high molar concentrations of sugars is likely at play, physical changes on the cotton surface, which became encrusted with solid sugar

within 24 h of deployment of high sugar solutions, also hindered feeding.

Of the four sugars, the disaccharides outperformed glucose and fructose. Maltose is especially interesting because it occurs in male German cockroach tergal gland secretions, along with maltotriose and other maltose derivatives (Nojima et al. 2002). These sugars are offered to females during courtship and thus serve as highly effective prenuptial phagostimulants, arresting the female while the male attempts copulation. Nevertheless, largely for economic reasons, we chose to develop and field evaluate a prototype bait consisting of 1% boric acid and 0.5 M sucrose. In addition to serving as a phagostimulant, the relatively high sucrose concentration also retards microbial growth and therefore significantly extends the interval between successive refilling of the baits in highly infested settings. Dethier (1976) stated that "next to water, sucrose is the most universally acceptable compound." Indeed, boric acid solutions containing sucrose have proved successful against other structural pests, including pharaoh ants (Klotz et al. 1997a) and Argentine ants (Klotz et al. 1998). Sucrose solutions containing  $\leq 1.0\%$  boric acid also resulted in 90% reduction in brood and workers of the red imported fire ant, *Solenopsis invicta* Buren (Klotz et al. 1997b).

Dispenser-contained liquid formulations of boric acid potentially could serve as effective alternatives to conventional cockroach management. Such dispensers would use  $\approx 1\%$  boric acid, representing a substantial reduction in active ingredient compared with dust ( $\approx 100\%$  [AI]) and solid bait (30–50% [AI]) formulations. The addition of inexpensive and readily accessible sugars can enhance the efficacy of boric acid, further reducing active ingredient concentration. The benefits of this approach include reducing risk of exposure to harmful dusts or more toxic insecticides that are typically used in cockroach control. Ultimately, acceptance of these baits will depend upon efficacy and ease of use. We have evaluated the efficacy of liquid formulations of boric acid for German cockroach population management in confinement swine production. Their efficacy is comparable with that of boric acid dust or residual applications of cyfluthrin wettable powder formulations (Gore et al. 2004).

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