Assimilation and Toxicity of Boron in the Argentine Ant (Hymenoptera: Formicidae)[1]

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Abstract Assimilation of boron by Argentine ants, *Linepithema humile* (Mayr), fed boric acid—sucrose water solutions was affected by the addition of sorbitol. Concentrations of 10 and 20% sorbitol reduced boron assimilation by 38 and 67%, respectively, thereby significantly reducing the toxicity of the solutions. The effect of solution pH on mortality indicated that the ionized borate molecule was less toxic than the neutral boric acid species. Electron microscopy revealed that ants fed low concentrations of boric acid (0.5%) show gross abnormalities in the microvilli and cells lining the midgut.

Key Words Argentine ants, *Linepithema humile,* baits, boric acid, sorbitol

Boron compounds have been used as insecticides since the middle 1800's for cockroaches (Ebeling 1995) and the early 1900's for ants (Rust 1986). Our research with Argentine ants, *Linepithema humile* (Mayr), has demonstrated that the oral toxicity of bait formulations with boric acid, borax, and disodium octaborate tetrahydrate is a function of the boron equivalents (Klotz et al. 2000). Formulation may affect feeding preference, and thus toxicity, but only by diminishing or enhancing consumption thereby affecting the ant's boron load. The mode of action of boron is not clear (Cochran 1995, Rust 1986).

More thoroughly understood is the biological activity of boron in plants. At low levels it is a micronutrient whose uptake (Bellaloni et al. 1999) and distribution in certain plants is facilitated by the presence of sugar alcohols (Hu et al. 1997). In species of *Prunus, Pyrus,* and *Malus* boron forms complexes with sorbitol which increases its mobility (Brown and Hu 1996). The water soluble sorbitol-boron-sorbitol complex is translocated to plant tissues via phloem where it plays a key structural role in cell wall formation as a pectin-boron complex (Hu et al. 1996). In a related manner, the Merck Index (Budavari 1989) states that sorbitol is used to "enhance absorption of vitamins and other nutrients in pharmaceutical preparations." Sorbitol's interaction with boron in plants and its use in medicine suggested to us that it might increase absorption of boron by ants when incorporated in a bait.

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Our objectives in this study were to: (1) determine the oral toxicity and boron load of ants feeding on boric acid—sucrose water baits, formulated with sorbitol; (2) determine the effect of solution pH on the relative toxicity of boric acid; and (3) use electron microscopy to examine the histological effects of low concentrations of boric acid on the ant's gastrointestinal tract. Results of this study may help us to better understand the biological activity of boron in ants and to develop more effective bait formulations with boron for pest control.

Materials and Methods

Toxicity tests. Argentine ants from colonies reared and maintained in the laboratory as described by Hooper-Bui and Rust (2000) were used in these tests. The ants were provided water but no food for 2 d prior to bait exposure. Concentration-mortality was determined using procedures described by Klotz et al. (1998). To formulate baits, crystalline boric acid (99% [AI], Sigma, St. Louis, MO) was dissolved in 25% (wt:vol) sucrose-deionized water to produce 0.5 and 1.0% boric acid solutions. Similarly, sorbitol and boric acid were dissolved in 25% sucrose water solutions to produce two concentrations of sorbitol (10 and 20%) in 0.5% boric acid. An additional set of bait solutions of 1.0% boric acid adjusted to pH 4, 5, 6, 7, 8, 9, and 10 with HCl or NaOH were prepared. Bait solutions were added to cotton plugs inside small Gelman Petri dishes (50 mm diam 9 mm) (Fisher, Ann Arbor, MI) containing 10 worker ants. Treatments and controls (25% sucrose water) were replicated 10 times. Bait solutions were available continuously to the ants for the duration of the test. Cumulative mortality was recorded daily until all ants died.

Preference tests. Binary choice tests were conducted in the field to determine rates of consumption by ants of sucrose water solutions with and without sorbitol. Two side-by-side feeding stations were attached to ten trees to measure differential consumption of 25% sucrose water versus 25% sucrose water + 10% sorbitol. Feeding stations consisted of 50 ml capped centrifuge tubes, with a 2 cm diam hole drilled through the 3.2 cm cap. A porous plastic material (WeedBlock, Easy Gardener, Waco, TX) was centered over the top of the centrifuge tube filled with each solution and the cap screwed down to secure it in place. Bait stations were inverted and taped side-by-side to the trunks of trees containing foraging trails of Argentine ants. The ants were allowed to feed on the solutions for 24 h after which the amount consumed in each of the tubes was determined gravimetrically.

Boron load analysis. The concentration of boron in the ants was determined using a colorimetric procedure that had been developed for plant tissue and soils (John et al. 1975). Groups of dead ants from the oral toxicity tests described above were air dried and weighed in ceramic crucibles. The groups of ants were ashed in a muffle furnace at 500°C, cooled, and the residue dissolved in 5.0 ml of 1.0 M HCl. Boron analysis was accomplished using azomethine-H, which forms a colored complex with boric acid. Color intensity was measured at 420 nm wavelength using a spectrophotometer (Milton Roy Spectronic 1001) and compared to standard solutions of 0, 0.1, 0.25, 0.5, 1.0, 2.0, and 3.0 mg B/L prepared in the acid. The detection limit for this procedure was better than 10 mg/kg (dry weight of ants).

Electron microscopy. Argentine ants were collected from a citrus grove on the Riverside campus of the University of California (Riverside, CA). The ants were provided water but no food for 1 d prior to bait exposure. They were then fed 0.5% boric acid in 25% sucrose water or 25% sucrose water (controls). After 24 h, ant midguts were dissected and fixed for 2 h in 0.1M sodium cacodylate buffer containing 4% glutaraldehyde, 4% paraformaldehyde and 0.05% $CaCl_2$ (pH 7.2). The midguts were washed several times in buffer and post-fixed 2 h in 0.1M sodium cacodylate buffer containing 1% osmium tetroxide, 3% potassium ferrocyanide and 0.05% $CaCl_2$ (pH 7.2). The midguts were again washed in buffer, dehydrated in a graded ethanol series and embedded in spurr resin. Uranyl acetate (3%) dissolved in 70% ethanol was used for 2 h during dehydration. Sections were cut on a Sorvol Mt2 ultramicrotome and post stained with uranyl acetate and lead citrate. The sections were observed in a Philips CM 300 transmission electron microscope. Ten control and 15 treated ants were sectioned and viewed.

Statistical analysis. Mortality data were corrected with Abbott's (1925) formula and analyzed by probit analysis (Raymond 1985) to determine median lethal times (LT_{50}) for each concentration. Binary choice tests to determine feeding preference for different solutions were analyzed using a paired *t*-test (StatView 1992).

Results and Discussion

Ants fed 25% sucrose water with boric acid plus sorbitol assimilated less boron than ants fed the same solution without sorbitol (Fig. 1). Rather than enhancing assimilation of boron, sorbitol appears to have complexed the boron, allowing it to be passed through the digestive tract and excreted. Thus, the greater the sorbitol content in the bait, the greater the LT_{50} of the ants because less boron is available for absorption.

Preference tests were conducted to determine if the lower toxicity of the boric acid-sucrose water solutions with sorbitol might be due to reduced consumption. Based on consumption rates of 25% sucrose water with and without sorbitol, in a side-by-side binary choice test, there was no significant preference by the ants for either solution (paired *t*-test, −1.37; df = 9, $P = 0.20$). In additional choice tests using arenas, *L. humile* showed no preference for 25% sucrose solutions or 25% sucrose with 5, 10, or 20% sorbitol. Workers did not feed on solutions, however, consisting of sorbitol alone. Sorbitol, therefore, is not a feeding deterrent nor is it a phagostimulant. Vander Meer et al. (1995) tested nine sugar alcohols, including sorbitol, as phagostimulants for the red imported fire ant, *Solenopsis invicta* Buren. Only myo-inositol showed slight phagostimulant activity.

Boric acid is highly permeable to plant cells unless it is complexed with polyols, sugar alcohols which occur in certain species, and used to transport boron in the phloem (Brown and Hu 1998). Species of plants with sorbitol exhibit considerable boron mobility versus other vascular plants containing sucrose, which exhibit much less boron transport (Brown and Hu 1998). It is noteworthy that sucrose did not have the same effect on ants as sorbitol. Argentine ants fed two different concentrations of sucrose water (10 and 25%) with 0.5% boric acid had similar LT_{50}s (Klotz et al. 1998). Apparently sucrose does not have the proper configuration of hydroxyl groups to complex with boron so that the boric acid is free to be absorbed. Thus, the uncomplexed boric acid is the more toxic form of boron.

In a second experiment, bait solutions of varying pH were tested for their relative toxicity (Fig. 2). The toxicity of the boric acid dropped significantly at pH values 9 and 10. This is attributed to the change in solution species as a function of pH. At high pH (above 9), boric acid is converted to the borate ion ($B(OH)_4^{-1}$), which appears to be

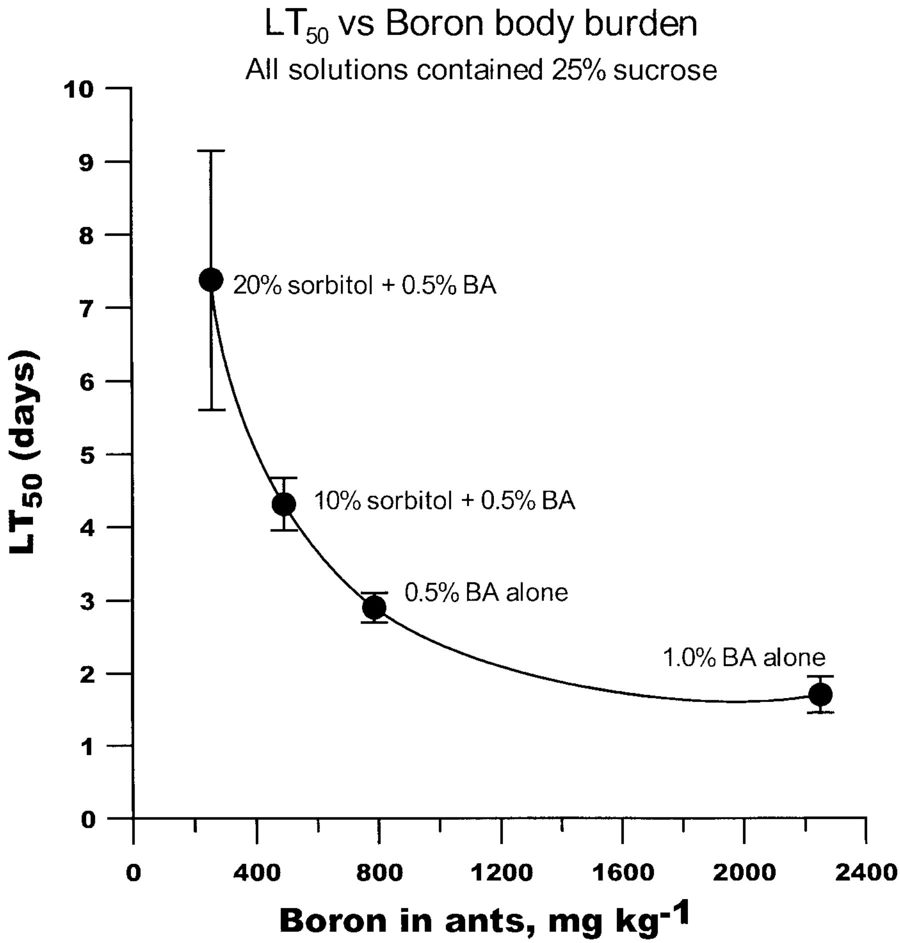

Fig. 1. The relationship between LT_{50} and the concentration of boron in the ants at death for boric acid—sucrose baits containing sorbitol.

less toxic than the neutral boric acid molecule ($B(OH)_3°$). The ionization constant for boric acid can be written as follows (Weast and Astle 1980):

$$B(OH)_3° + H_2O \leftrightarrow B(OH)_4^{-1} + H^+ \quad \log K = -9.14 \qquad [1]$$

The percent of the neutral species ($B(OH)_3°$) as a function of pH is plotted in Figure 2, showing the relationship between relative toxicity and degree of boric acid ionization. We speculate that the borate anion is more easily excluded by cell membranes, whereas the uncharged species can pass unrestricted.

Major differences were seen between the midgut lining of boric acid-treated and untreated Argentine ants. After 24-h exposure to 0.5% boric acid in 25% sucrose water, over 40% of the specimens examined contained damaged cells. Some epi-

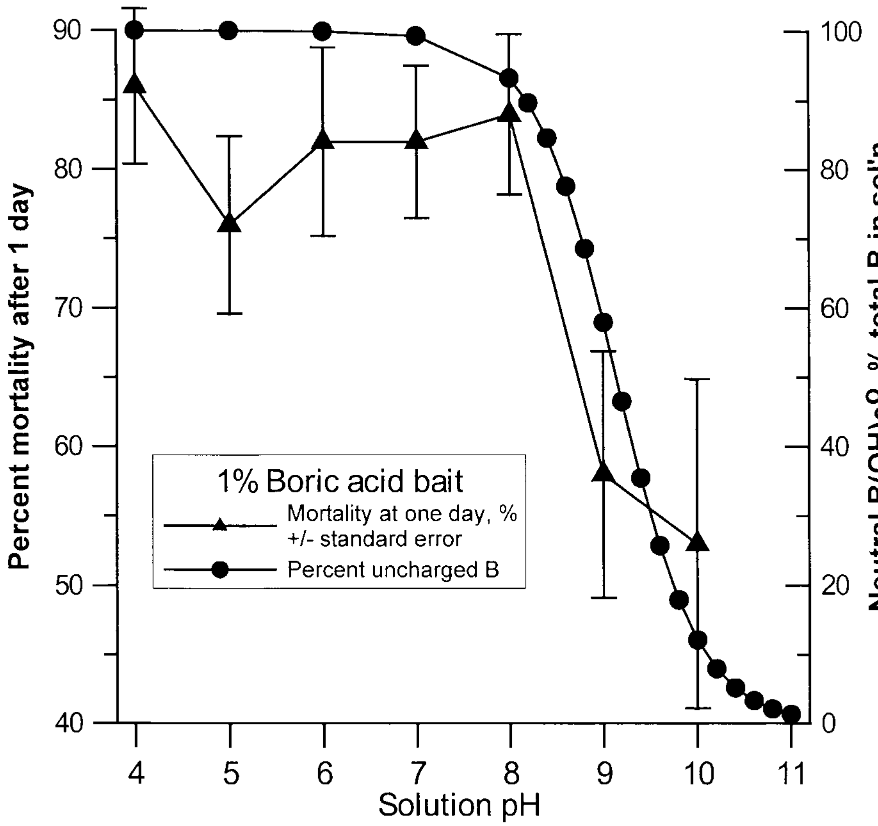

Fig. 2. The effect of pH on the relative toxicity of boric acid bait solutions. Also plotted is the percent of uncharged boric acid species as a function of pH. This curve was calculated using Eq. (1) in the text.

helial cells were completely destroyed, and the microvilli of the midgut lining were grossly affected (Fig. 3a,b). The midguts of untreated ants appeared normal with cellular organelles and microvilli intact (Fig. 3c,d). Cochran (1995) found major disruption of the foregut tissue in German cockroaches, *Blattella germanica* (L.), exposed to 2% boric acid in 2% sucrose water. Little or no damage was found in the gastrointestinal tract posterior to the foregut. He concluded that the probable cause of mortality was starvation brought on by destruction of the foregut. In contrast, our results may be due to the specialized digestive tract of ants, which is adapted for collection, storage and trophallaxis of liquids.

The mode of action of boric acid against ants remains unclear, but its toxic effects may derive from its ability to create complexes with organic functional groups (Woods 1994), not unlike its interaction with sorbitol in plants. For example, the borate anion forms a trigonal complex with inositol, an important component of cell membranes (Williams and Atalla 1981) and a boronate complex of the ribose group of NAD results

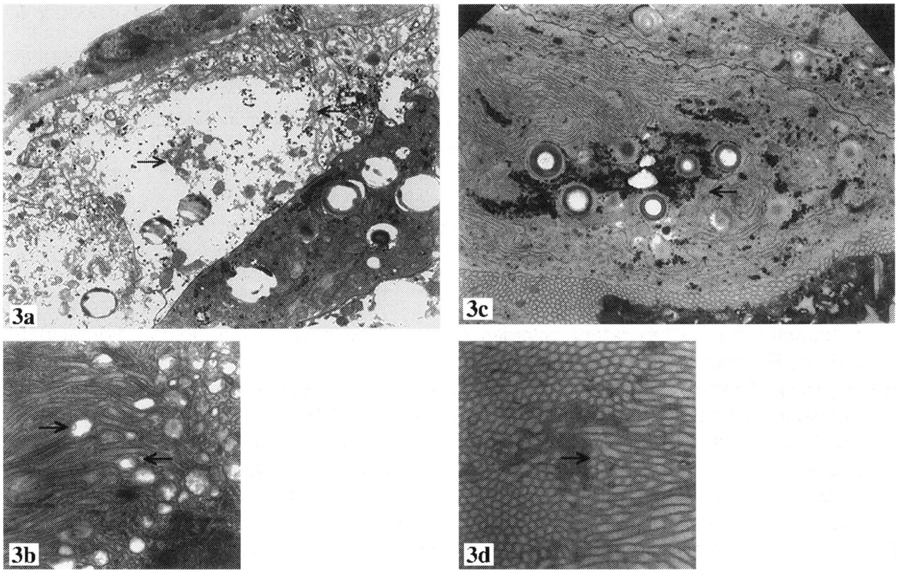

Fig. 3. Cells from the midgut of ants fed 0.5% boric acid in 25% sucrose water, showing in (a) damage to the cytoplasm of the cell (15,000X) and (b) swelling of the microvilli (21,350X). Cells from the midgut of ants fed 25% sucrose water without boric acid showing in (c) normal cytoplasm (14,150X), and (d) normal microvilli (27,430X).

in inhibition of this key enzyme (Woods 1994). Indeed Woods (1994) states that enzyme interactions, such as inhibition, are probably the most important feature of boron chemistry with respect to health effects. In addition to the toxicity caused by these possible molecular interactions, we found significant damage also being caused by boric acid on the cellular level.

In conclusion, we found that the assimilation of boron by Argentine ants feeding on boric acid-sucrose baits was affected by the addition of sorbitol. Sorbitol complexes with boric acid preventing its assimilation, thereby reducing its toxicity. At the tissue level, 0.5% boric acid causes gross abnormalities in microvilli and complete destruction of cells lining the midgut.

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