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Research

Effectiveness of Boric Acid by Ingestion, But Not by Contact, Against the Common Bed Bug (Hemiptera: Cimicidae)

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Abstract

Boric acid has been used as an insecticide in the successful control of agricultural, public health and urban pests long before the advent of synthetic organic pesticides. Boric acid products, formulated as dusts, sprays, granular baits, pastes, gels, and liquids, are widely available to consumers and pest management professionals, especially to control pest infestations within homes. Boric acid dust is commonly used against bed bugs (*Cimex lectularius* L. [Hemiptera: Cimicidae]), but its efficacy has not been demonstrated. We evaluated the efficacy of boric acid as an ingestible and residual contact insecticide on bed bugs, and compared its efficacy on the German cockroach (*Blattella germanica* L. [Blattodea: Ectobiidae]) which is known to be susceptible to boric acid by both routes. Dose–response studies of 0–5% boric acid in blood demonstrated that ingested boric acid caused rapid mortality at concentrations of ≥2%, and even 0.5% and 1% boric acid caused 100% mortality, albeit at a slower time course. In contrast, bed bugs survived contact with high concentrations of boric acid dust. Smaller boric acid particles did not increase mortality of either unfed or recently fed bed bugs. The same boric acid products were effective at causing mortality of German cockroaches by both contact and ingestion. We thus conclude that although boric acid is an excellent candidate active ingredient for an ingestible bait formulation, residual applications of dust or spray would be ineffective in bed bug interventions.

Key words: chemical control, boric acid, Public Health Entomology, Urban and Structural Entomology, bed bug

Bed bug infestations continue to increase worldwide, and in the United States, all 50 states are plagued with their presence. Bed bugs exert a range of health risks in infested homes, hotels, or other indoor settings. Although they have not been shown to vector any diseases to humans, in a laboratory setting bed bugs are able to successfully transfer the pathogen in Chagas disease, *Trypanosoma cruzi*, to mice (Salazar et al. 2015), and *T. cruzi* persists across bed bug molts (Blakely et al. 2018). Bed bug bites can also cause severe dermatologic reactions in some people, and excessive scratching can lead to secondary infections (Rossi and Jennings 2010). A recent study showed that bed bug feces contain large amounts of histamine, which accumulates in household dust and has the potential to elicit adverse respiratory and dermatological responses (DeVries et al. 2018). Other adverse effects include increased anxiety, depression (Susser et al. 2012), and avoiding public places for fear of bringing bed bugs home (Goddard and de Shazo 2012). Residents in infested homes often over-use and misuse 'do-it-yourself' insecticides in efforts to eradicate bed bug infestations (Jacobson et al. 2011).

Current interventions to eliminate bed bug infestations include the application of residual insecticides as sprays or dusts, spatial heat treatments, and targeted steam or freezing treatments to areas such as the seams of mattresses. All these approaches have significant constraints and shortcomings. Residual insecticides have become an increasing concern due to the rapid evolution of resistance to pyrethroids (Davies et al. 2012) and neonicotinoids (Romero and Anderson 2016), the major classes of insecticides labeled for use against bed bugs. Bed bugs have evolved diverse mechanisms of resistance, including target site mutations that reduce sensitivity to insecticides (Zhu et al. 2010, Booth et al. 2015), expansions of detoxifying enzyme families' high expression of which supports resistance (Adelman et al. 2011, Benoit et al. 2016), and cuticular thickening, which is a general resistance mechanism to a wide range of residual, contact insecticides (Koganemaru et al. 2013, Benoit et al. 2016, Lilly et al. 2016). Resistance can trigger recurrent applications of marginally effective insecticides, ultimately resulting in greater human exposure to insecticide residues (Gangloff-Kaufmann et al. 2006). Although heat treatments are often successful (Pereira et al. 2009), the treatment protocol requires complex,

specialized, and expensive equipment, and there is potential of damage to belongings due to the high temperatures required to eradicate bed bugs (Kells and Goblirsch 2011).

Desiccant dust formulations, such as diatomaceous earth and silica gel, have been incorporated into bed bug interventions. However, application of these products has practical constraints, as does their efficacy. Bed bugs also exhibit behavioral avoidance of repellent dusts, reducing direct contact which is required for efficacy of diatomaceous earth, dinotefuran, and silica gel (Agnew and Romero 2017). In most cases, the dust alone is not sufficient to control infestations. Studies have shown that in combination with CO_2 (Aak et al. 2017), alarm pheromone (Benoit et al. 2009), or when formulated as an aerosol spray (Akhtar and Isman 2016), the efficacy of silica and/or diatomaceous earth dusts can be substantially improved.

Boric acid has been deployed successfully for controlling a wide range of pests in agricultural, structural, and public health settings (Durmus and Buyukguzel 2008, Bicho et al. 2015, Lachance et al. 2017). In combination with a phagostimulant, often in a simple water-based formulation, boric acid was shown to be effective at reducing populations of ants, cockroaches, and mosquitoes (Klotz et al. 1998, Xue and Barnard 2003, Gore et al. 2004, Gore and Schal 2004, Naranjo et al. 2013, Wang et al. 2017). Boric acid is also commonly used in more complex gel formulations against cockroaches, and even as a feeding deterrent when used at high concentrations to protect wood against termites (Tsunoda 2001).

The mode of action of boric acid is still equivocal, but presumably includes destruction of the lining of the foregut (Cochran 1995) and neurotoxicity (Habes et al. 2006). Early studies also demonstrated that boric acid caused mortality in German cockroaches (*Blattella germanica* L. [Blattodea: Ectobiidae]), even when their mouthparts were sealed, suggesting that it penetrated the cuticle (Ebeling et al. 1975). Major advantages of boric acid as component for a yet to be developed ingestible bed bug bait would be its low toxicity to humans and pets compared with other insecticides (Weir and Fisher 1972; Murray 1995, 1998), low cost, and high solubility in water. Moreover, despite a century of use, there is no evidence of insect resistance to boric acid (Cox 2004), possibly related to its inorganic properties.

Previously, we performed a proof of concept study showing that several neuroactive insecticides were highly effective when ingested by bed bugs in blood using an artificial feeding system (Sierras and Schal 2017). The lethal dose by ingestion was about 45-fold lower than the dose required in topical applications. Similar studies with ivermectin and moxidectin ingestion by humans and mice also showed mortality of bed bugs that ingested the treated blood (Sheele et al. 2013, Sheele and Ridge 2016).

Boric acid is commonly used in do-it-yourself control of household pests, including in bed bug–infested homes (Moore and Miller 2009). We are not aware, however, of any formal assessment to determine whether boric acid is effective for controlling bed bugs. Our objective was to evaluate the efficacy of boric acid against bed bugs by ingestion and residual application, for its possible incorporation into a bed bug bait and integrated pest management program. We demonstrated that although boric acid was highly efficacious on bed bugs by ingestion, it had marginal efficacy as a dust in residual applications. We used the German cockroach in a comparative study to show that the same boric acid was effective on *B. germanica* by both routes of delivery.

Materials and Methods

Insects

Colonies of *Cimex lectularius* L. (Hemiptera: Cimicidae) (Harold Harlan [HH] = Fort Dix strain, collected in 1973; insecticide susceptible) and *B. germanica* (Orlando Normal = American Cyanamid strain, collected >70 yr ago in Florida; insecticide susceptible) were maintained in separate incubators at 27°C, ~50% RH, and on a 12:12 (L:D)-h regime. Only adult males were used in all experiments because their physiological state is less variable and less dependent on reproduction status than females. Moreover, our previous investigation with ingestible insecticides showed no major differences in their toxicity on first-instar nymphs and adult males (Sierras and Schal 2017). All experimental insects were held in the same conditions. Bed bug colonies were fed defibrinated rabbit blood using the artificial feeding system described in Sierras and Schal (2017). German cockroaches were fed rodent chow (Purina No. 5001 Rodent Diet, PMI Nutrition International, Brentwood, MO) and given water as described in Wada-Katsumata et al. (2013).

Experiment 1: Feeding Assays With Bed Bugs

Technical grade boric acid (≥99% purity, Fisher Scientific, Fairlawn, NJ) was dissolved in rabbit blood to 0, 0.5, 1, 2, 3, 4, and 5% (wt/vol). A 1% boric acid solution is 0.16 M. Ten adult male bed bugs were collected randomly from the colony and placed in a 4-ml glass vial with a plankton screen top (BioQuip Products, Rancho Dominguez, CA) through which they could feed. A paper substrate (strip of manila folder) in the vial reached the screen, so bed bugs could reach the feeding membrane. Each group of 10 bugs was given 30 min to feed, but after 15 min a glass Pasteur pipette was used to remix the blood-boric acid solution, as in Sierras and Schal (2017). Only fully fed (engorged) males, determined visually as detailed in Sierras and Schal (2017) (Fig. 1A–C), were kept and mortality was monitored daily for 7 d. Three replicate vials (N = 30 males) were used per treatment.

Experiment 2: Residual Contact Assays With Bed Bugs

Boric acid (BORiD, Waterbury Companies, Waterbury, CT) was weighed (Sartorius 1712 MP8 Silver Edition, Sartorius Lab Instruments, Goettingen, Germany) and distributed into 60- × 15-mm Petri dishes (Falcon, Corning, NY). To determine whether 1) higher concentrations of boric acid are more effective, and 2) fed bed bugs are more susceptible to boric acid dust because their intersegmental membranes are more exposed after they feed, we exposed bed bugs to 13.8 mg of boric acid per dish, representing the label rate (0.48 mg/cm^2) or 138 mg per dish representing 10 times the label rate; controls (no boric acid) were also included. Bed bugs placed in the assays were either fully fed or starved 7–10 d. Each replicate consisted of 9–10 adult males per dish and a total of 118 starved and 120 fully fed adult male bed bugs were used. Bed bugs remained in their treatment dishes for 24 h and then bed bugs from each replicate dish were placed into a clean Petri dish. Mortality was monitored daily for 14 d.

We also hypothesized that boric acid particle size might affect mortality by influencing the interaction with intersegmental membranes and spiracles. To reduce the particle size, ~0.35 g of boric acid (BORiD) was placed into 2-ml tubes (FastPrep, MP Biomedicals, Santa Ana, CA) with 1.44-mm ceramic beads (MP Biomedicals) and ground in a reciprocating homogenizer (FastPrep24 5G, MP Biomedicals) with four 30-s cycles (total of 2 min). Bed bugs were assayed on the ground boric acid as described above, but only at the label rate (0.48 mg/cm^2), and mortality was monitored daily.

We quantified the size of boric acid particles to confirm that grinding reduced their size distribution. Boric acid particles were scattered on conductive carbon tape on a scanning electron microscopy (SEM) stub, coated with gold/palladium (Hummer 6.2 Sputtering System,

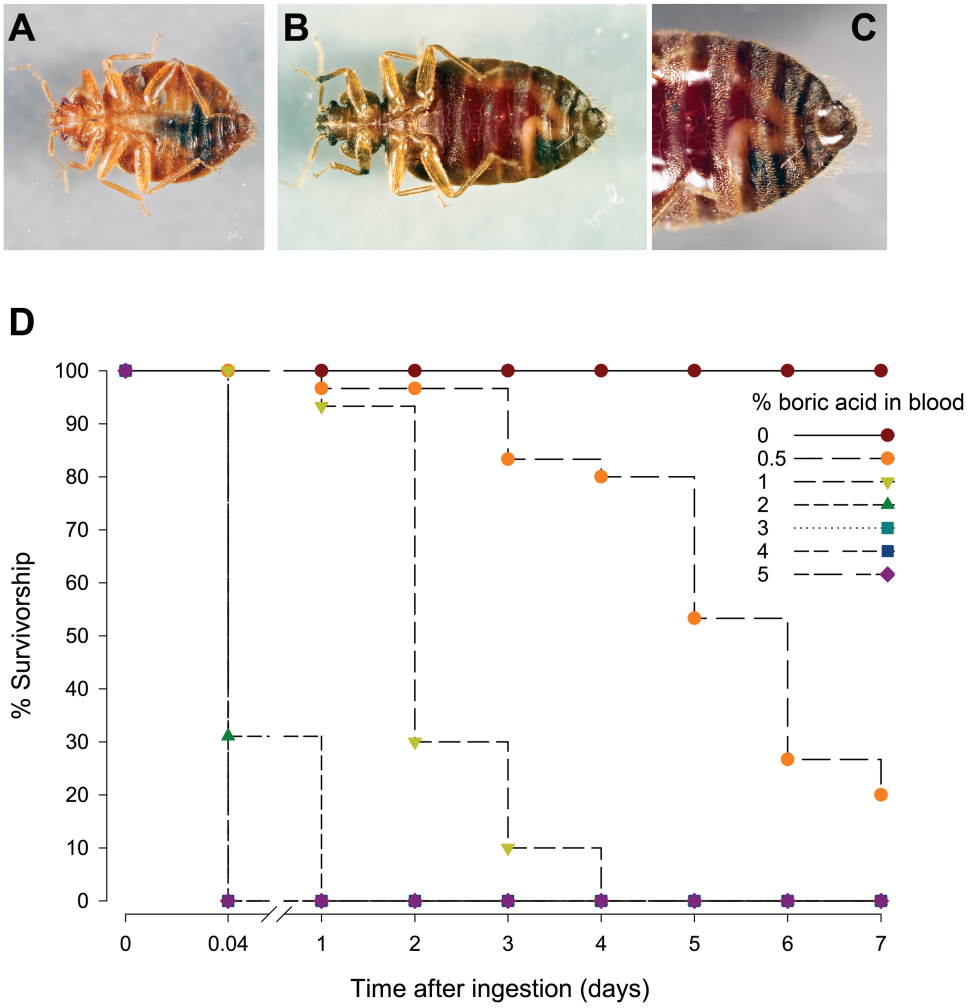

Fig. 1. Unfed (A) and fully engorged (B) adult male *C. lectularius*, showing the exposed abdominal intersegmental membranes in fed bed bugs (C). (D) Kaplan–Meier survivorship of adult male *C. lectularius* fed once to repletion on various concentrations of boric acid in defibrinated rabbit blood. Each treatment group consisted of three replicates (n = 24–30 per group, Table 1). Mortality was recorded for 7-d postingestion.

Table 1. Percentage of fully engorged adult male bed bugs that ingested different concentrations of boric acid in defibrinated rabbit blood and amount of boric acid they ingested

Concentration of boric acid in blood (%)	Number in treatment group (n)	Number fully engorged[a]	% fully engorged	Ingested boric acid (µg/adult male)[b]
0	30	30	100	0
0.5	30	30	100	19.5
1	30	30	100	39
2	30	29	97	78
3	30	27	90	117
4	30	25	83	156
5	30	24	80	195

[a]Adult male bed bugs were given 30 min to feed.

[b]Calculated based on the observation that each adult male bed bug consumes 3.9-µl blood (Sierras and Schal 2017).

Anatech, Hayward, CA), and observed by SEM in high vacuum (JSM-5900LV, JEOL, Peabody, MA). We also observed the cuticular surface of adult male bed bugs exposed to boric acid dust and control males that were not exposed to boric acid. Three treatment groups included three males each: 1) control males; 2) males exposed to nonground boric acid; and 3) males exposed to ground boric acid. Bed bugs were exposed to 13.8-mg boric acid in Petri dishes (60 × 15 mm; 0.48 mg/cm^2) for 24 h. Each bed bug was then killed by decapitation and prepared for SEM. We arbitrarily defined a 100-µm radius around the right spiracle on the ventral side of the seventh abdominal sternite and quantified the size of all boric acid particles in this region (0.03 mm^2). ImageJ (National Institutes of Health, Bethesda, MD) was used to quantify the size of boric acid particles to which bed bugs were exposed, as well as the boric acid particles that adhered to the cuticular surface of treated males.

Experiment 3: Residual Contact Assays With Cockroaches

Adult male German cockroaches (9–11 d old) were used. To control for their ingestion of boric acid, two treatment groups were used: one with glued mouthparts and a control treatment. Both groups were immobilized briefly with CO_2 and placed on ice. The mouthparts of some males were then glued (Loctite Super Glue ULTRA Gel Control, Henkel Corporation, Rocky Hill, CT) to prevent ingestion of boric acid after grooming. The control group received the same treatment but mouthparts were not glued. Cockroaches were placed

into Petri dishes that were 100 × 20 mm (Falcon, Corning, NY) in replicates of 3–6 males per dish (n_{total} = 26–41 males per control or treatment group). Males were then exposed to boric acid dust at the label rate (0.48 mg/cm^2, 38.4 mg per dish) and mortality was monitored daily.

Experiment 4: Injection Assays With Bed Bugs

To assess the toxicity of boric acid to bed bugs, we injected it directly into the hemocoel. Boric acid was solubilized in phosphate-buffered saline (PBS, 0.008-M sodium phosphate, 0.002-M potassium phosphate, 0.14-M sodium chloride, and 0.01-M potassium chloride, Fisher Scientific, Fairlawn, NJ) to obtain a 5% stock solution, and diluted in PBS to the desired concentrations for five treatment groups, including PBS-only controls. All adult males (20 per treatment group; n= 100) were starved 4 d prior to injection. Bed bugs were immobilized briefly with CO_2 and placed ventral side up on ice, and 0.2 or 0.5 µl of the PBS solution was injected through the intersegmental membrane using a custom-made glass capillary attached to a 31-gauge syringe (Hamilton, Reno, NV), delivering 0, 1, 10, or 25 µg boric acid/bed bug. Bed bugs were placed individually into Petri dishes (60 × 15 mm) lined with filter paper (Whatman #1, 55 mm, Fisher Scientific, Fairlawn, NJ). Treatment groups were then placed into plastic containers with a vented lid and a moist paper towel lined the bottom of the container. Mortality was recorded daily for 7 d.

Statistical Analysis

Survivorship analysis was used for residual, ingestion, and injection assays using a Kaplan–Meier and log-rank test performed in SAS (2012). A Sidak adjustment was used for log-rank tests for multiple comparisons. Kolmogorov–Smirnoff test was used to analyze differences in the particle size distributions of ground and nonground boric acid in Petri dishes and on adult male bed bugs after 24 h of exposure.

Results

Mortality of Bed Bugs After Ingesting Boric Acid

A dose–response study was conducted with different concentrations of boric acid in defibrinated rabbit blood, using an artificial feeding system. Bed bugs readily accepted low concentrations of boric acid, as 97% fed to repletion on up to 2% boric acid (Table 1). However, at boric acid concentrations of ≥3%, the number of bed bugs that fully engorged (Fig. 1B) declined to 80% at 5% boric acid. We also calculated the amount of boric acid each adult male ingested based on previous findings that each adult male consumes 3.9 µl when fully engorged (Sierras and Schal 2017), and at the lowest (0.5%) and highest (5%) concentrations, each bed bug consumed 19.5- and 195-µg boric acid, respectively (Table 1).

None of the control bed bugs fed defibrinated blood died in the 7-d assay, but all bed bugs that ingested 1 to 5% boric acid died within 4 d (Fig. 1D). There were significant differences among all groups (log rank, χ^2 = 264.76, df = 6, P < 0.0001) and between the control group (0% boric acid) and all other treatment groups (0.5, 1, 2, 3, 4, 5%; P < 0.0001 for all comparisons). Pairwise comparisons revealed significant differences between the 0.5% treatment group and the 2, 3, 4, and 5% groups (P < 0.0001 for all, Sidak adjustment). The lowest concentration of boric acid, 0.5%, killed 50% of the males by day 5 and only 20% survived to 7 d. The highest concentration of boric acid that was readily ingested, 2%, killed 70% of the bed bugs within 1 h and 100% within 24 h after ingestion.

Mortality of Bed Bugs After Contact With Boric Acid Particles

We found low mortality in assays with unfed adult male bed bugs exposed to the label rate (0.48 mg/cm^2) of boric acid dust distributed in a Petri dish. Only 33% of the bed bugs that were exposed to boric acid particles for 24 h died within 14 d (Fig. 2). We then

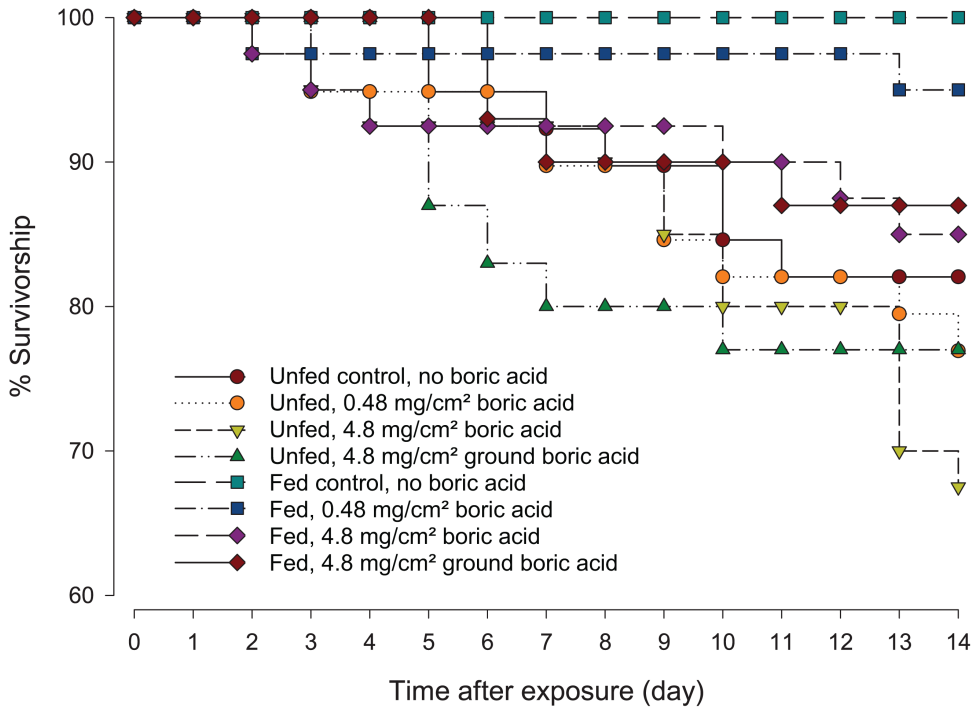

Fig. 2. Kaplan–Meier survivorship of adult male *C. lectularius* that were exposed to different concentrations of boric acid dust for 24 h and monitored for mortality for 14-d postexposure. The label rate of boric acid dust is 0.48 mg/cm^2. Each treatment group consisted of 3–4 replicates (n = 30–40 bed bugs per treatment).

examined the effect of larger amounts of boric acid and feeding status to assess if fed bed bugs with exposed intersegmental membranes were more susceptible than unfed bed bugs (Fig. 1A–C). We predicted that 1) higher concentrations of boric acid would be are more effective, 2) fed bed bugs would be more susceptible to boric acid dust because their intersegmental membranes are more exposed after they feed, and 3) smaller particles of boric acid dust would be more effective.

Although there were significant differences among the treatment groups (χ^2 = 22.51, df = 8, P = 0.004; log-rank test), a 10-fold higher concentration of boric acid dust (4.8 mg/cm^2) increased mortality of unfed males by only 10% (Fig. 2). Likewise, we found no significant differences between the fed treatment groups and the unfed bed bugs. Fed bed bugs exposed to the highest concentration (10× label rate) exhibited only 15% mortality, and unfed bed bugs exposed to the same concentration experienced a similar mortality of only 33% after 14 d (Fig. 2). Therefore, both increasing the boric acid concentration and exposing the intersegmental membranes did not increase the susceptibility of bed bugs to boric acid.

Finally, to determine whether smaller particles of boric acid would be more effective at causing mortality in bed bugs, we ground boric acid to smaller particles to which bed bugs were exposed for 24 h. First, we confirmed by SEM that the grinding procedure reduced the particle size distribution of boric acid dust. The size of the unground particles (n = 338, $\bar{x}$ = 56.3 µm, median = 20.7 µm) was significantly reduced after grinding (n = 340, $\bar{x}$ = 14.2 µm, median = 9.1 µm) (P < 0.0001; Kolmogorov–Smirnov; Fig. 3A). Approximately 20% of the unground boric acid particles were >100 µm, whereas the largest particles after grinding were 35 µm or smaller (Fig. 3A).

SEM observations revealed that boric acid particles on bed bugs represented only a fraction of the distribution of boric acid particles to which bed bugs were exposed. Thus, when bed bugs were exposed to nonground boric acid, small particles were disproportionately represented on their cuticle (n = 150, $\bar{x}$ = 7.3 µm, median = 5.9 µm), compared with the overall distribution of dust particles with $\bar{x}$ = 56.3 µm, showing that large boric acid particles were excluded from the cuticle (Fig. 4B and C). Bed bugs that were placed in Petri dishes with ground boric acid dust also picked up

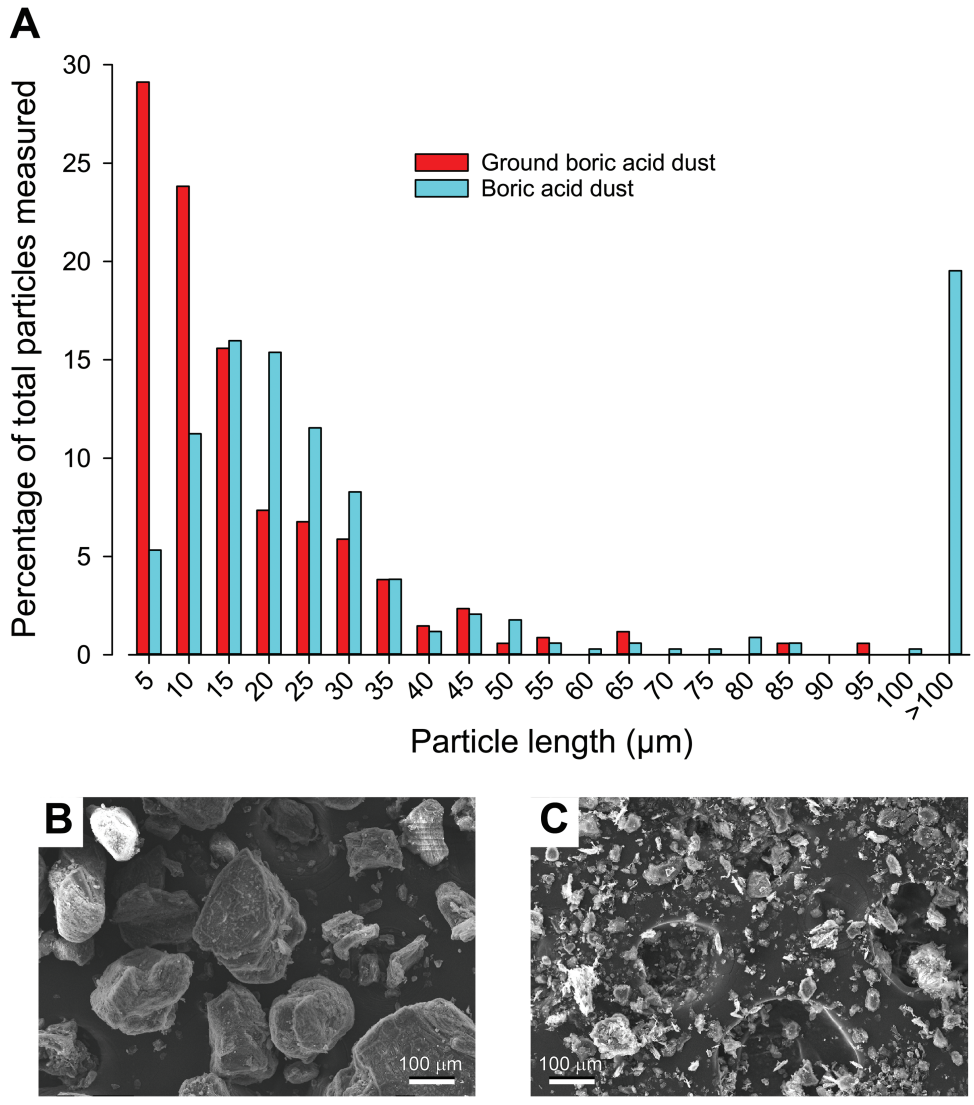

Fig. 3. Particle size distributions of nonground (n = 338 particles) and ground (n = 340 particles) boric acid dust (A). Boric acid particles were measured from SEM images (magnified 130×) as the longest axis of each particle. These images represent similar images used for measurements. Particle sizes were binned in 5-µm increments and each bin was represented as a percentage of the total number of particles measured for nonground (B) and ground (C) dust.

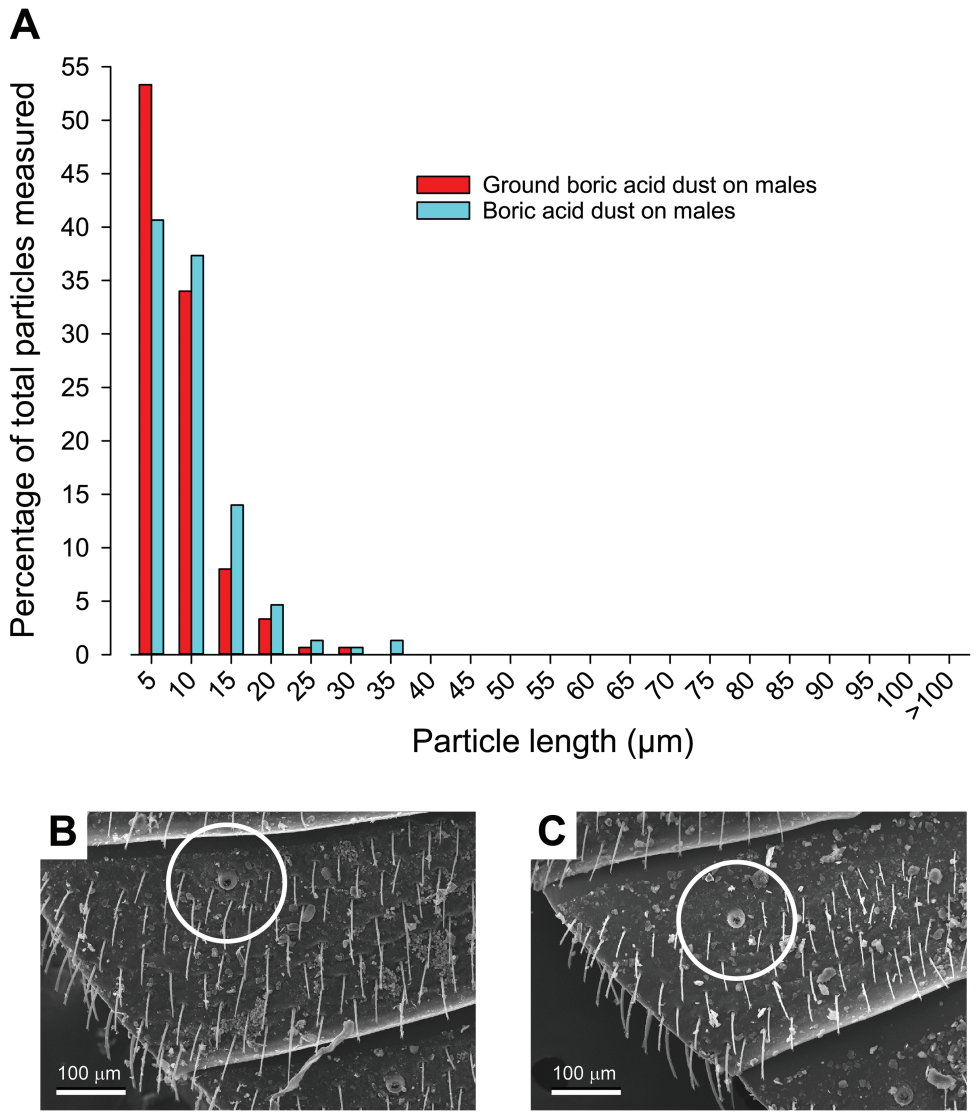

Fig. 4. Particle size distributions of nonground (three replicates; n_{total} = 150) and ground (3 replicates; n_{total} = 150) boric acid dust, to which bed bugs were exposed in Petri dish assays, quantified from the cuticular surfaces of male bed bugs after 24 h of exposure (A). Boric acid particles were measured from SEM images (magnified 200×) as the longest axis of each particle. These images represent similar images used for measurements. Particle sizes were binned in 5-µm increments and each bin was represented as a percentage of the total number of particles measured for nonground (B) and ground (C) dust on the cuticular surface. Measurements of particles were conducted in an area of 100-µm radius around the spiracle on the right ventral side of the seventh abdominal segment (as shown in white).

smaller particles (n = 150, $\bar{x}$ = 6.1 µm, median = 4.7 µm) than the overall distribution of ground boric acid with $\bar{x}$ = 14.2 µm (Fig. 4A). Although the size distributions of the nonground and ground particles on the cuticle converged onto much smaller sizes of $\bar{x}$ = 7.3 µm and $\bar{x}$ = 6.1 µm, respectively, they remained significantly different (P = 0.04; Kolmogorov–Smirnov).

However, reducing the size of boric acid particles did not improve their efficacy at causing bed bug mortality (Fig. 2). The label rate of boric acid that was applied to Petri dishes (0.48 mg/cm^2) was ineffective at causing mortality within 2 wk after exposure for all combinations of ground and nonground boric acid, and fed and unfed bed bugs. More than 85% of the fed bed bugs survived exposure to nonground or ground boric acid at both the label rate and 10× the label rate. Survivorship also did not differ significantly between the nonground and ground boric acid treatment groups for either fed or unfed males, even at 10× the label rate ($\chi^2 \leq 7.23$, $P \geq 0.22$; Fig. 2).

Mortality of Cockroaches After Contact With Boric Acid

The effectiveness of boric acid on *C. lectularius* by ingestion, and its marginal effectiveness as a dust prompted us to use *B. germanica* as an overall control because it is known to be highly susceptible to boric acid by both ingestion and contact. Because cockroaches ingest the dust that they groom off their body, we sealed their mouthparts to prevent ingestion of boric acid. Control cockroaches with sealed mouthparts that were not exposed to boric acid did not experience more mortality than control cockroaches with functional mouthparts (χ^2 = 6.25, P = 0.07; Fig. 5). However, mortality was significantly higher in both treatment groups that were exposed to boric acid than in both control groups with glued or functional mouthparts ($\chi^2 \geq 29.68$, $P < 0.0001$ for all comparisons). There was no significant difference between the two treatment groups that were exposed to boric acid dust (χ^2 = 1.46, P = 0.77), with 98–100%

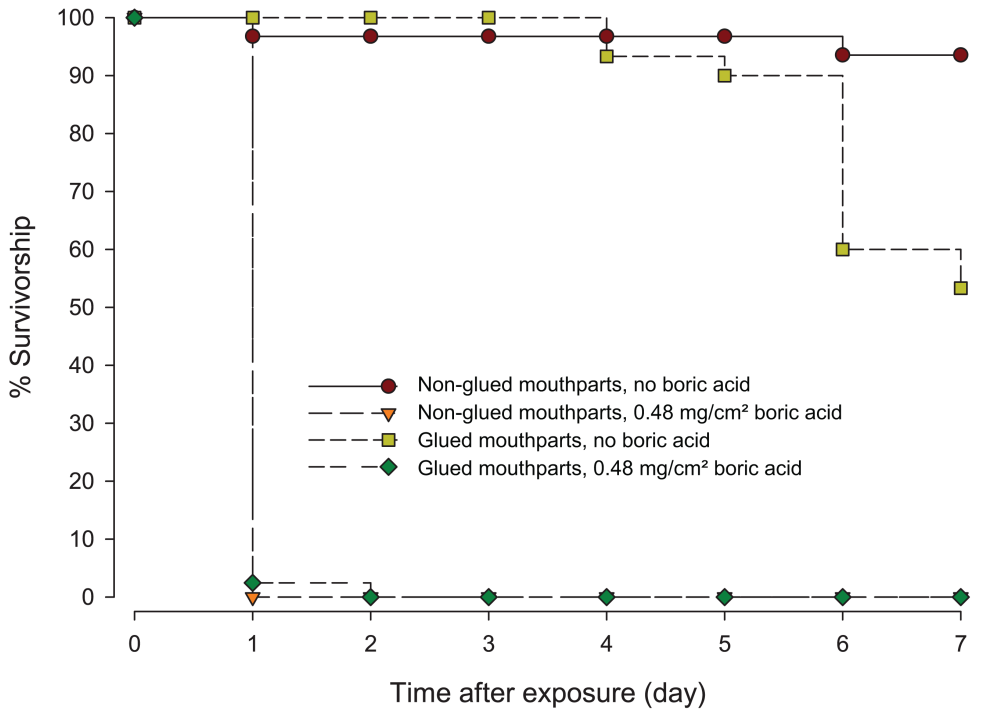

Fig. 5. Kaplan–Meier survivorship of adult male *B. germanica* that were exposed in Petri dishes to 0.48 mg/cm² (label rate) of boric acid (BORiD) for 24 h. Treatments included glued mouthparts to prevent cockroaches from ingesting boric acid. Mortality was monitored for 7-d postexposure. $n = 26–41$ per treatment group.

mortality within 24 h, indicating that contact with boric acid dust caused significant mortality in *B. germanica*.

Mortality of Bed Bugs After Injection of Boric Acid

Boric acid was highly effective at causing mortality when injected into the hemocoel, a procedure that bypassed the digestive system (Fig. 6). We injected two different PBS controls (0.2 and 0.5 µl) and three doses of boric acid in PBS: two doses (1 and 10 µg boric acid per bed bug) that were less than the lowest effective concentration ingested by bed bugs (0.5%, corresponding to 19.5-µg boric acid per bed bug; Table 1) and one dose (25-µg boric acid per bed bug) that was greater than the 19.5-µg exposure through ingestion. There were significant differences among the treatment groups (log rank, $\chi^2 = 71.50$, df = 4, $P < 0.0001$) (Fig. 6). The lowest dose, 1 µg per bed bug, did not differ significantly from the two control groups ($P > 0.28$), but the two higher doses resulted in greater mortality than the control groups ($P \leq 0.0014$). However, the 1 and 10 µg per bed bug injections did not differ significantly from each other ($P = 0.05$), exhibiting 40 and 64% mortality at day 7, respectively. Injections of 25-µg boric acid per bed bug, however, resulted in 95% mortality by day 7 (Fig. 6). These findings indicate that boric acid delivered to the hemocoel is toxic to bed bugs, that passage through the alimentary system is not necessary for mortality to be expressed, and that boric acid dust is somehow prevented from penetrating the bed bug cuticle.

Discussion

Overall, our results show that although boric acid is highly effective on bed bugs by ingestion, it has marginal efficacy by contact. In ingestion assays, we observed that relatively low concentrations of boric acid, up to 2% in rabbit blood, were readily ingested, as indicated by a high percentage of bed bugs fully engorging on the boric acid containing blood. Higher concentrations of boric acid deterred some bed bugs from feeding, yet 80% of the bed bugs accepted 5% boric acid, the highest concentration that we tested. Concentrations of ≥2.25% boric acid in a 10% sucrose solution were shown to deter feeding in house flies (Hogsette and Koehler 1994) and ≥ 6.25% boric acid in dry-mixed and wet-mixed baits deterred German cockroaches from feeding (Strong et al. 1993). Deterrence of boric acid at high concentrations has prompted the use of borates to protect and preserve wood against termites (Kartal 2010, Han et al. 2012, Lopez-Naranjo et al. 2016) and wood-boring beetles (Robinson 1967, French 1969, Palanti et al. 2012).

Ingested boric acid was highly effective at causing bed bug mortality. Even a single bloodmeal of 0.5% boric acid, the lowest concentration we tested, killed 80% of the bed bugs in 7 d, and concentrations of ≥1% killed 100% of the bed bugs within 4 d. Studies that have evaluated boric acid as a component of ingestible baits have shown it to be highly effective. German cockroaches ingesting an aqueous solution containing 0.5–2% boric acid with common sugars experienced rapid population declines when tested in the laboratory (Gore and Schal 2004), and using similar concentrations in the field in a 0.5-M sucrose solution significantly decreased cockroach infestations in swine farms (Gore et al. 2004). Likewise, argentine ants are susceptible to 0.5% boric acid in a 25% sucrose in water solution (Klotz et al. 1998, Hooper-Bui and Rust 2000). Toxic sugar-bait solutions that contain 1% boric acid are effective at reducing blood-fed, gravid female mosquitoes by 98% within 48 h in laboratory tests (Xue and Barnard 2003) and in the field spraying 1% boric acid in a melon-derived solution, which was naturally attractive to both male and female mosquitoes, reduced *Anopheles gambiae* populations by 90% (Muller et al. 2010).

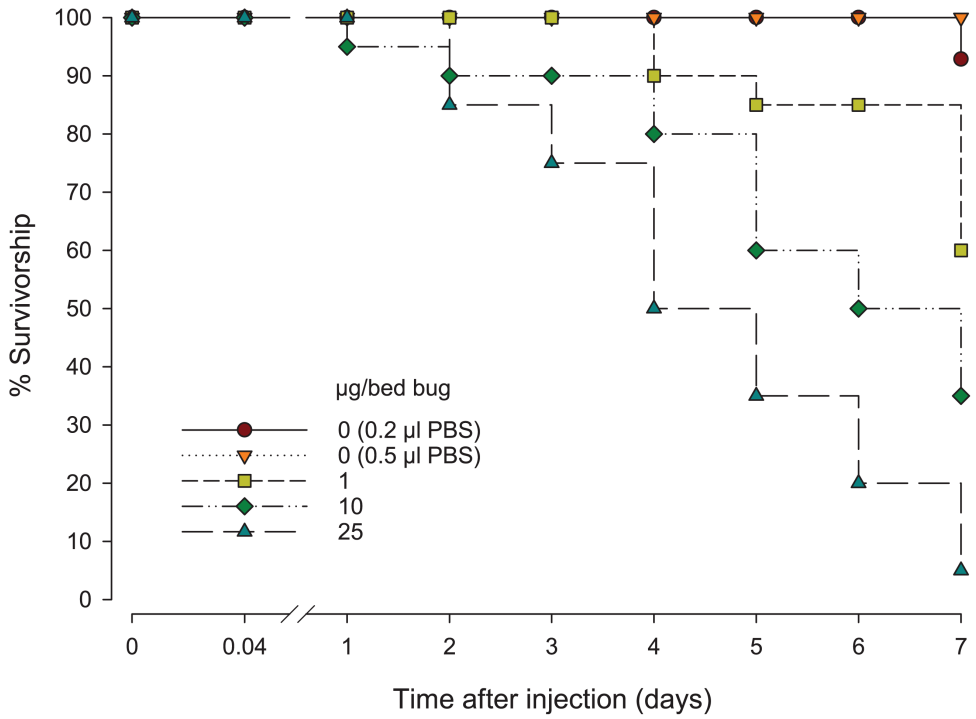

Fig. 6. Kaplan–Meier survivorship of adult male *C. lectularius* injected with various amounts of boric acid in PBS. Each treatment group consisted of 20 individuals. Mortality was recorded for 7-d postinjection.

Surprisingly, bed bugs were relatively unaffected by contact with boric acid dust. Even 24 h of continuous exposure to 10× the label rate of boric acid dust resulted in only 15 and 32.5% mortality, 14-d postexposure in fed and unfed bed bugs, respectively. Because the bed bug cuticle is covered with many setae that may prevent larger particles from contacting the cuticular surface, we suspected that smaller particles might adhere to and interact more with the cuticle and spiracles, thus increasing efficacy. Smaller particle size, however, did not improve the efficacy of boric acid dust. In fact, we found that regardless of the particle size distribution of boric acid dust applied to the dish, only small particles adhered to the bed bug cuticle. We further hypothesized that feeding status influences survivorship because the dust adheres to the abdominal intersegmental membranes, which are more exposed in engorged bed bugs. However, both groups experienced low mortality, with more fed than unfed bed bugs surviving the 10× label rate of boric acid.

Our results showed that the route of boric acid administration greatly affected its efficacy. Ingested boric acid was highly effective against bed bugs, whereas boric acid administered by contact was ineffective. This surprising finding prompted us to use the same boric acid materials on the German cockroach, *B. germanica*, which is known to be susceptible to boric acid by both ingestion and contact (Ebeling 1978). The same boric acid dust that was ineffective on bed bugs was highly effective on the German cockroach. Thus, it appears that this otherwise insecticide-susceptible strain of bed bugs possesses mechanisms that prevent boric acid from compromising or penetrating the cuticular barrier.

The mode of action of boric acid remains unclear. Its effectiveness on bed bugs by ingestion, but not by contact, suggests that it might specifically disrupt the digestive system, as suggested by Cochran (1995) for cockroaches. To experimentally bypass both the alimentary canal and cuticle, we injected boric acid into the hemocoel. Ebeling (1995) and Zurek et al. (2002) showed that boric acid caused high and faster mortality when injected into German cockroaches than when ingested. Boric acid was effective by injection on bed bugs as well. A minimal ingested dose of 19.5-µg boric acid killed ~80% of fully engorged bed bugs, and similarly, an injected dose of 25-µg boric acid killed ~95% of the bed bugs in 7 d. These results confirm that the mode of action of boric acid in bed bugs involves neither the cuticle (e.g., desiccation) nor the digestive system (e.g., starvation or tissue degradation). Rather, the adverse effects of boric acid on the insect are expressed after it enters the hemocoel and may potentially have neurotoxic effects on bed bugs, such as elevated glutathione *S*-transferases and reduced acetylcholinesterase activity observed in German cockroaches exposed to boric acid (Habes et al. 2006).

Ebeling et al. (1975) suggested an explanation for the differential mortality in cockroaches after exposure to boric acid dust, its ingestion in liquid, and injection. Boric acid dust that adheres to the cuticle is groomed off the body and ingested, but it remains in the foregut and takes a long time to penetrate the thick cuticle that lines the foregut. When dissolved in water, boric acid readily passes to the midgut and hindgut, from where it more quickly penetrates the hemocoel. Injection bypasses these barriers and exerts the fastest effects on the insects. Our observations with bed bugs are consistent with these suggestions, but with the added constraint that boric acid dust cannot be ingested by bed bugs. Interestingly, the rapid mortality we observed in bed bugs that ingested boric acid in a bloodmeal may be related to the adaptation of hematophagous insects to rapidly transfer water from the bloodmeal to the hindgut for excretion. This process may also facilitate the rapid transfer of dissolved boric acid out of the foregut.

Boric acid is commonly used to control a wide variety of urban pests, including bed bugs. A 2011 study found that boric acid was available as a 'do-it-yourself' (DIY) product in 70% of 120 stores surveyed in New York City, and it was the most common nonspray

product found (Horton et al. 2011). Indeed, dust formulations of boric acid are effective against other crawling insects, including cockroaches, silverfish, and fleas (Hinkle et al. 1995, Rust and Dryden 1997, Wang and Bennett 2009). We have observed that many residents use boric acid dust against bed bugs as well (A.S., personal observations). But the low morality we observed in bed bugs exposed for 24 h of continuous contact with boric acid dust indicates that interventions using boric acid dust would not be effective against bed bugs. Drivers for such misguided use of boric acid dust for bed bug control likely include lack of efficacy data documenting its inefficacy, high cost of alternative methods of bed bug control, and the wide availability of boric acid dust to consumers.

Our research considered only adult male bed bugs of the Harold Harlan population, which is susceptible to all insecticides and maintained in the laboratory for ~45 yr. Future studies should evaluate females, nymphs, and recently field-collected populations. Also, only fully engorged individuals were monitored for lethal effects following the ingestion of boric acid. Further research should examine the sublethal effects of ingested boric acid and its effects on partially fed individuals. Furthermore, future research should investigate why boric acid is ineffective against bed bugs as a residual insecticide, and strategies that might correct this deficiency. For example, spatial heat can synergize the effects of boric acid in cockroaches and beetles (Ebeling 1995). Because spatial heat is a common though expensive bed bug intervention, its interaction with boric acid dust should be further investigated.

A major challenge in developing effective bed bug interventions is to develop, validate, and implement a bed bug bait. Their piercing-sucking mouthparts make this task particularly challenging for hematophagous arthropods. In previous research, we showed that several neuroactive insecticides were much more effective by ingestion in an artificial feeding system than by topical application (Sierras and Schal 2017). Ivermectin and moxidectin administered by ingestion to humans and mice also showed systemic effects and mortality of bed bugs that ingested the treated blood (Sheele et al. 2013, Sheele and Ridge 2016). Boric acid appears to be an excellent candidate for a bed bug baiting system, and its solubility and stability in water, low mammalian toxicity, low cost, and no evidence of resistance to it in any insect species make it a particularly appealing candidate. The research we report here was conducted with boric acid dissolved in rabbit blood. Phagostimulants in blood likely maximized blood intake and possibly obscured some deterrence at higher concentrations of boric acid. However, Romero and Schal (2014) showed that the addition of ATP to water could simulate bed bugs to ingest large amounts of water. Furthermore, heat itself strongly stimulates close-range attraction and feeding in bed bugs (DeVries et al. 2016), so the combination of ATP, heat, and boric acid could be important components of a bed bug baiting system.

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