Ingested Boric Acid Effect on the Venom Chemistry of *Solenopsis invicta* Buren (Hymenoptera: Formicidae)[1]

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Abstract During a field evaluation of a boric acid bait against the red imported fire ant, *Solenopsis invicta* Buren, it was observed that workers of intoxicated colonies produced stings with less toxic effects compared with workers from healthy colonies. The present study was undertaken to investigate the effect of boric acid on the levels of piperidine alkaloids in ant venom was investigated. Exposure to boric acid for a period of 4 wks resulted in significant changes in both concentrations and ratios of the piperidine alkaloids. The concentration of piperidine alkaloids was significantly lower in colonies treated with the boric acid bait. Thus, boric acid suppresses the production of piperidine alkaloids in red imported fire ants.

Key Words red imported fire ant, solenopsin, piperidine, allergen, bait

The red imported fire ant, *Solenopsis invicta* Buren, was introduced in Alabama (Buren et al. 1974) and is rapidly spreading throughout the southeastern and southwestern US (Callcott and Collins 1996), affecting more than 20 million people and stinging about 2.5 million persons per month. It has been calculated that 16% of people stung by the ant show an allergic reaction to the venom and need medical treatment, and in a few instances, results in death (Paull et al. 1983). Allergic response to the venom can be determined by the levels of IgE antibodies in the patient (Paull et al. 1983) and from skin tests (Strom et al. 1983). Hoffman et al. (1988) isolated 4 allergens from the venom of these fire ants and named them as Sol i I, Sol i II, Sol i III, and Sol i, IV. In this same work, Hoffman et al. (1988) also reported that based on amino acid composition, these allergens were structurally different from each other. They reported that the IgE responses to the allergens were not related to each other. The RAST test study done by Butcher et al. (1988) showed that the allergic effect of the venom is due to its proteins, which constitute approx. 0.1% of the venom weight (Baer et al. 1979) indicating that protein components of the venom of *S. invicta* are different from those venoms of other fire ants in the *Solenopsis* genus, such as *S. richteri* (Forel), *S. xylony (*McCook), and *S. germinate* (Fabricius).

One of the methods for controlling fire ants is by the use of baits (Williams 1994). Boric acid has been widely used as a bait active ingredient due to its low toxicity to mammals and slow mode of action (Williams 1994, Klotz and Moss 1996). The toxicity of

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boric acid as a bait active ingredient has been studied previously (Williams 1994, Erwin 1996, Klotz and Moss 1996, Klotz et al. 1997), and its efficacy in controlling fire ant populations under field conditions when applied in a systematic mound-by-mound treatment of large areas was reported (Rojas et al. 2005). During field evaluation studies of boric acid baits against *S. invicta*, it was observed that workers of intoxicated colonies produced stings of diminished noxious effects as compared with workers from healthy colonies (M.G. Rojas, unpubl. data). We hypothesized that venom production was somehow affected by boric acid ingestion. The objective of the study reported herein was to determine the effect of boric acid on *S. invicta* venom composition when ingested by workers.

Materials and Methods

Red imported fire ant colonies containing workers, queens, and brood were collected from Mount Bayou (Bolivar Co.) and Stoneville (Washington Co.), MS. The samples were placed in Sterilite® 11.4 L, rectangular plastic boxes (Sterilite,Townsend, MA) with the open edges lined with an 8-cm wide band of fluon (AD-1, Northern Products, Inc. Woonsocket, RI) to prevent them from escaping. The bottom of each box was lined with absorbent paper towels to provide the ants with a substrate suitable for humidity regulation in the colony. A source of water was provided via a 30- cm long X 3.5-cm diam glass tube plugged with cotton. Ants were provided either with gel Drax Nutrabait® (Waterbury Companies, Inc., Waterbury, CT) containing 5% boric acid, or the Drax Nutrabait® formulation (Rojas et al. 2005) without boric acid as a control. Three g of gel bait was applied daily directly on top of each ant mound. Colonies were maintained at 25°C ± 2 and 16: 8 L:D cycle. Workers were sampled from colonies 4 wks after the treatments were started and at regular intervals of 7 d for a total of 6 samples of each treatment and control groups.

Venom extraction. Ant workers were randomly selected from treated and untreated colonies, and venom was extracted using 2 methods. The first method consisted of extracting venom droplets from 15 workers of each group using a 1-µL capillary tube with the aid of a stereo microscope as reported by Blum et al. (1958). The abdomen of the ant was held and squeezed with a pair of fine forceps to promote venom excretion. A 1-µL capillary glass tube was used to stroke the lower abdomen of the fire ant to expose the stinger, which then excreted the venom. The 1-µL tube was gently placed next to the stinging apparatus to collect the venom at the tip of the tube. Then, a gastight 100-µL glass syringe (part No. 1710, Agilent Technologies, Palo Alto, CA), cleansed thoroughly with hexane, was used to place 30 µL of the hexane in a 100-µL conical crimp style glass vial (part No. 9,480, Alltech Associated Inc., Deerfield, IL). The 30 µL of hexane was marked at its meniscus on the vial in case there was any evaporation of hexane whereas performing the venom extraction procedure. After gathering about 100 droplets of venom from several fire ant workers, the capillary tube was dipped in the hexane and stirred to dissolve any venom residue on the sides of the tube and its contents were blown out using a micro rubber bulb. Once the sampling was completed, each vial was capped with a Teflon crimp style top seal (part No. C4011 - 1A, National Scientific Co., Rockwood, TN). The 12 vials were placed into an auto-sampler tray to be analyzed by GC/MS (Hewlett Packard 6,890 GC/5973 MSD, Agilent, Technologies, Palo Alto, CA). After the samples were run by the system, it was determined that the samples were, in fact, too weak to provide reliable results. The second method consisted of separating the abdomen from the head and thorax to collect the ovipositors along with the venom glands. As stated above, fire ant workers were randomly selected to collect ovipositors and venom glands. As above, 30 µL of hexane was placed into each 100-µL crimp top vial and marked at the meniscus. Under a stereo microscope and with the help of 2 sets of fine tip forceps, the fire ant's ventral part of the abdomen was positioned and tightly held with one of the forceps to face upwards to display its stinging apparatus. A second pair of forceps was used to physically cut at the base of the abdomen to preserve the ovipositor and venom gland. After the abdomen was removed, it was immediately placed inside a 2-ml plastic centrifuge tube containing the 30 µL hexane. Five different-sized fire ants per sample were randomly used from each treatment and control to reach a sample size of 100 mg. Tubes with samples were centrifuged at 14,000 rpm for 10 min. Then, the content of the tubes was separately transferred to 0.45-µm, nylon, mini-uniprep syringeless filter units and crushed as the contents passed through the nylon membrane. Filtered samples were subsequently transferred to glass vials with the help of a gastight 500 µL glass syringe (part No. 1750, Agilent, Technologies, Palo Alto, CA). Any loss of hexane was then replaced to achieve a final volume of 50 µL. The vials were then crimped and analyzed as previously mentioned. Fifteen equal samples were used for the treatment and 15 for the control.

Chemical analyses. The extracted solutions were analyzed with a gas chromatograph/mass spectrometer (GC/MS) (Hewlett Packard, 6,890 GC/5973 MSD, Agilent, Technologies, Palo Alto, CA) equipped with a 7,683 auto injector and a VF-5ms, WCOT fused silica [250.00 µm and film thickness of 0.25 µm capillary column (Varian CP8944, Varian, Palo Alto, CA)]; ultra-pure helium was used as the carrier gas with a 35.1 ml/min flow. One µl sample was automatically injected at 250°C and 27.75 KPa (3.59 psi) using inlet split mode with a split ratio of 50:1, a split flow of 31.8 ml/min and total helium flow of 35.1 ml/min. Oven temperature was programmed from 60°C to 215°C with a constant gradient of 15 degrees/min and from 215°C to 280°C at 8 degrees/min, to avoid ester coelution, with a final temperature hold of 10 min. The mass spectrometer was tuned using the manufacturer's ATUNE.U program. Acquisition mode was set to scan with a temperature of 280°C and 3 min solvent delay, 1470.6 resultant EM voltage. In addition, the following scan parameters were used: low mass: 15, high mass: 550, threshold: 150, sample #: 2, A/D samples = 4, plot 2 low mass: 50, plot 2 high mass: 550; MSZones for MS quad: 150°C maximum 200°C, MS source: 230°C, maximum 250°C. The GC/MS was controlled with HP Chem-Station (Model No.6890N/5973N, Agilent Technologies, Palo Alto, CA). Peaks on the chromatograms were identified by comparing retention times and MS spectra to those reported in Wiley7N, 7th edition MS spectral library (Product No. G1035B, Agilent Technologies, Palo Alto, CA) and comparing their MS spectra of standards.

Statistical analysis. Data consisting of peak area resulting from integration plots for each peak and from each sample were used to compare "abundance" of each of the compounds between treatments. These data were analyzed in 2 ways: (1) by analysis of variance of "abundance" comparing the means with Student's *t*-test; and (2) by nonparametric means using the Wilcoxon paired sample test (Zar 1999). Both procedures were performed using JMP statistical software (SAS Institute 2008).

Results and Discussion

Brand et al. (1972) characterized the venom of fire ants as 6-methyl piperidine alkaloids, and later this was confirmed by other studies (Lai et al. 2008, Chen et al. 2009, Chen and Fadamiro 2009a, Chen and Fadamiro 2009b).The five peaks present in our chromatograms were identified as piperidine derivatives; peaks 2 and 3 corresponded to solenopsin A and B, respectively (Fig. 1). Exposure to boric acid for a period of 4 wks resulted in significant changes in the concentrations and ratios of piperidine compounds (Fig. 1). The concentration of piperidine alkaloids was significantly higher in ant workers from the control group compared with ant workers treated with boric acid ($F = 19.18$; $df = 1, 103$; $P < 0.0001$). Statistical analysis of individual compound concentration showed that 5 compounds (represented by peaks 1 - 5) were significantly

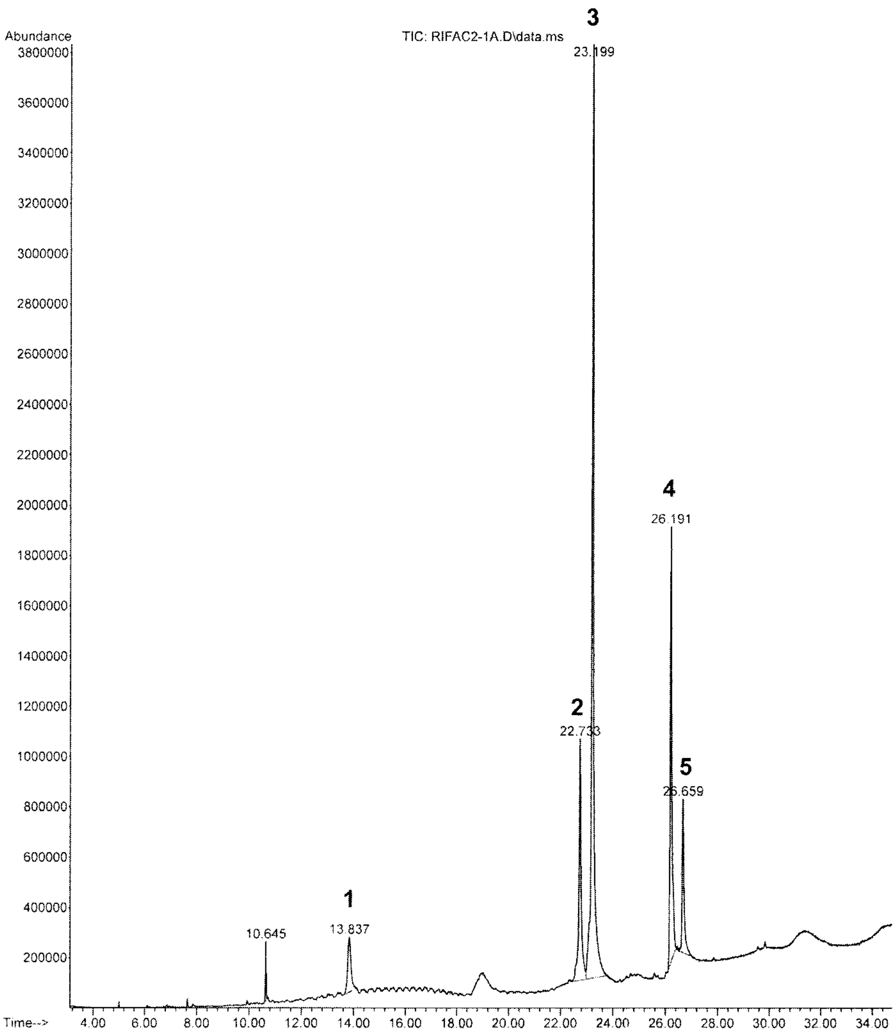

Fig. 1. Chromatogram of *S. invicta* venom from workers feeding on a bait devoid of boric acid (control) showing the 5 peaks compared. Peaks 2 and 3 correspond to solenopsin A and solenopsin B, respectively.

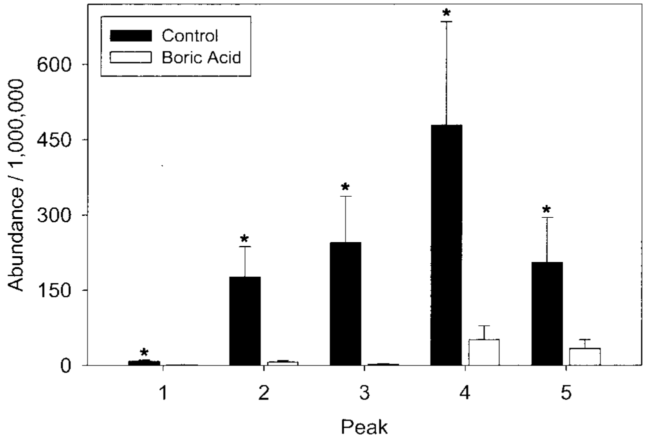

Fig. 2. Comparison of mean abundance of 5 compounds represented by peaks 1 - 5 from the venom of *S. invicta* fed with a bait devoid of boric acid (control) in black, and bait with 5% boric acid in white. Brackets represent standard error of the mean. Bars with star are significantly higher based on Wilcoxon paired-sample test at α = 0.05.

higher in the control group as compared with the boric acid treated group. Both analyses of peaks 1 - 5, parametric (F = 9.52, 10.64, 9.29, 5.65 and 4.6; df = 1, 19; P = 0.0061, 0.0041, 0.0066, 0.0281 and 0.0451, respectively) and nonparametric (Z = 3.24, 3.24, 3.87, 2.74, and 2.24; df = 19; P = 0.001, 0.0012, 0.0001, 0.0062, and 0.0251, respectively) yielded similar results (Fig. 2). Nonparametric analysis was performed because significant deviations from normality were detected in the parametric analysis.

Mundy and Bjorklund (1985) reported a procedure for the synthesis of solenopsin A via a novel bicyclic ketal fragmentation reaction. Sodium tetrahydridoborate was used as catalytic agent in this process. We propose that boron cations from boric acid ingested by the worker ants may be competing for active sites of intermediary compounds and playing a role in the natural synthesis of these compounds. Further research on the subject is still in progress.

Fire ant venom is known to possess herbicidal, bactericidal, and fungicidal activities, and one of its functions is to contribute in maintaining the health of the colony (Schmidt 1986, Taber 2000). Fire ant venom alkaloids inhibit the germination of conidia of the entomopathogenic fungi, *Beauveria bassiana* (Balsamo), *Metarhizium anisopliae* (Metsch.), and *Paecilomyces fumosoroseus* (Wize) (Storey et al. 1991). It is conceivable that a colony intoxicated with boric acid may be more susceptible to entomopathogenic fungi and thereby rendered more vulnerable to microbial control. Future research will address this possibility.

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