

## Effects of Short-Chain Fatty Acids on House Crickets, Orthoptera: Gryllidae

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### Abstract

*The effectiveness of short-chain fatty acids (SCFAs) as a biodegradable insecticide was examined on house crickets, Acheta domesticus (L.). Mature (0.3523g ± 0.0813 g) and immature (0.0191g ± 0.0013 g) crickets were sprayed for 40 s which delivered 1.47 mL over a 78.5cm<sup>2</sup> surface. Butanoic acid (1.36 M, pH 4.0) was the most lethal treatment and killed 100% of the immature and adult crickets within 10 minutes whereas the controls had no mortality for 24 hours. The LC<sub>50</sub> values for propionic, butanoic, and pentanoic acid were 1.10M, 0.44M and 0.26M, respectively, for immature crickets. The LC<sub>50</sub> values for propionic, butanoic, and pentanoic acid were 0.82M, 0.57M and 0.16M, respectively, for adult crickets. The SCFAs (propionic, butanoic, and pentanoic) at their highest concentrations induced a drop in hemolymph pH from 8.24 to 7.86, 7.24 and 7.88, respectively. These three SCFAs were effective in killing both immature and adult house crickets and show promise as a “green” insecticide.*

**Key Words:** house cricket, fatty acid, control.

### 1.0 Introduction

House crickets, *Acheta domesticus* (L.), are house pests during certain times of the year. They are omnivorous scavengers that feed on almost any organic material and when present in large numbers, are known to cause damage to clothing, silks, vegetables and fruits. The key to managing house crickets inside buildings is exclusion, however, when this fails, boric acid baits can be effective. Fatty acid salts have been shown to be effective in killing and repelling cockroaches (Baldwin, Koehler, & Pereira, 2008) and causing mortality in a wide variety of insects (Puritch, 1975, 1981). Specifically, propionic acid is effective against some stored-grain pests (Germinara, Rotundo, & De Cristofaro, 2007). Fatty acids are naturally derived from plant oils and animal tallow (Puritch, 1981). Short-chain fatty acids (SCFAs) are effective in paralyzing and killing a wide range of organisms, such as fruit flies (Farine, Legal, Moreteau, & Le Quere, 1996; Legal, Moulin, & Jallon, 1999), and inactivating pig worm eggs (Butkus et al., 2011).

Toxicity of SCFAs at the cellular level has been attributed to acidification as the undissociated form enters the cell and releases its proton and thus leads to disruption of plasma membranes (Baronofsky, Schreurs, & Kashket, 1984), causing the accumulation of anions (Dashper & Reynolds, 2000) and the depletion of ATP supply as the cell tries to actively pump out the protons. The toxicity of saturated fatty acids has been shown to increase with an increase in chain length, peaking at C<sub>10</sub> and C<sub>12</sub> (Baldwin et al., 2008). The potential use of SCFAs as an outdoor insecticide is an environmental friendly, or “green” chemistry, process. Excess SCFAs will enter the soil where they would be neutralized by the soil or the soil could be sprayed with baking soda to amend this process. This would cause the acid to disassociate and become far less toxic since it could no longer cross a cellular membrane. SCFA can be biodegraded by various microorganisms (Aguilar, Casas, & Lema, 1995; Sheridan, Curran, & Dodd, 2003).

In the present study, the potential activity of three SCFAs (propionic acid, butanoic acid, and pentanoic acid) was investigated as an effective pesticide against the house cricket, using a modified bottle sprayer method.

## **2. Materials & Methods**

### **2.1 Insects**

House crickets, *Acheta domesticus*, were purchased from a commercial vendor (Sue's Zoo, New Paltz, and NY). Crickets were maintained in a cage at  $25.0 \pm 2.5^{\circ}\text{C}$  and an average humidity level of  $71 \pm 15\%$  RH for 24-96 hours before experimental trials. Dry food (Fluker's® High-Calcium Cricket Feed) and water (Fluker's® Cricket Quencher) were provided *ad libitum*. Harborage consisted of rolled corrugated cardboard. The average lifespan of *Acheta domesticus* in a laboratory setting is ~120 days at  $30^{\circ}\text{C}$  with maturity defined by the presence of wings and mature genitalia after molt (Lyn, Aksenov, LeBlanc, & Rollo, 2012). Based on these criteria, second, third and fourth instar immature (wingless and lacking ovipositors) and mature crickets were tested with average weights of 0.0191g ( $\pm 0.0013$  g) and 0.3523 g ( $\pm 0.0813$  g), respectively to show the effects of SCFAs on immature and mature cricket populations.

### **2.2 Preparations of short-chain fatty acid solutions**

The pH of the stock solutions of propionic acid,  $\text{C}_3\text{H}_6\text{O}_2$  (1.5 M), butanoic acid,  $\text{C}_4\text{H}_8\text{O}_2$  (1.36 M) and pentanoic acid,  $\text{C}_5\text{H}_{10}\text{O}_2$  (0.2 M) was adjusted to 4.0 with solid NaOH. The stock solutions were diluted with deionized water and the pH was confirmed to be 4.0. All solutions were stored at  $4^{\circ}\text{C}$  and warmed to room temperature before use.

### **2.3 Calibration of bottle sprayer using butanoic acid**

A modified bottle sprayer was used (Nansen, Hinson, Davidson, Vaughn, & Ghalralari, 2010) to spray crickets. The airbrush (Master MAS G22; www.amazon.com) was inserted into an open ended plastic bottle with a diameter of 12.7 cm and secured by clamps. A gas pressure regulator was attached to the in-house air supply which delivered air to the airbrush at  $18 \pm 2$  psi. A standard 100 mm petri plate ( $78.5 \text{ cm}^2$ ) was placed 23.5 cm from the airbrush. A calibration curve was generated to determine the volume of butanoic acid sprayed over time (data not shown).

### **2.4 Determination of the $\text{LC}_{50}$ for the short-chain fatty acids**

Immature and adult crickets in control and treatment groups were placed separately in 100 mm plastic petri dishes (five per plate) and chilled at  $4^{\circ}\text{C}$  for 10 minutes to reduce their mobility. Various concentrations of acids were sprayed onto the crickets for 40 s which released 1.47 mL of acid. After spraying, the crickets from two plates were transferred into glass petri dishes (150mm) containing food and water. The crickets were maintained at  $25^{\circ}\text{C}$  and high humidity with observations at 10 minutes and 24 hours post treatment. Crickets were assumed dead when there was no response to physical probing. Linear regression of the data was used to determine the  $\text{LC}_{50}$  of the acids for large and small crickets. Initial studies carried out showed the crickets were not affected by spraying with distilled water or acidified distilled water (data not shown).

### **2.5 Determination of cricket hemolymph pH**

Crickets were chilled on a BioQuip Laboratory Chill table (BioQuip Products, Rancho Dominguez, CA) at  $-10^{\circ}\text{C}$  until immobile (5-10 minutes). Hemolymph samples were obtained from mature crickets from cerci that were cut close to the abdomen. The abdomen was gently squeezed to expel hemolymph being careful not to squeeze so hard that gut contents were also expelled, this liquid would appear milky or greenish (Woodring, 1985). 15  $\mu\text{L}$  of clear liquid at the tip of the cercus was collected and then placed directly on a pH indicator strip (pH 5-10; ColorpHast® pH Indicator Strips, EM Science, Gibbstown, NJ) and read while still moist. The pH of 10 crickets was measured for each treatment. The pH measured for ten untreated cricket hemolymph was 8.24. Crickets which had been sprayed were rinsed with distilled water for 15 s, transferred to a clean petri dish and placed under a 150 watt lamp for approximately 2 minutes to allow excess liquid to evaporate. The crickets were placed on the chill table again before bleeding.

## **3. Results and Discussion**

The SCFAs induced a statistically significant drop (Student t-test,  $p < 0.01$ ) in blood pH of treated crickets whereas HCl did not (Table 1). An exposure of 5-10 minutes to HCl fumes was required to cause a slight reduction in blood pH and cause mortality.

This is consistent with previous research that demonstrated the injection of strong acid into the hemocoel caused only a slight decrease in hemolymph pH in *Schistocerca gregaria* (Phillips, Hanrahan, Chamberlin, & Thomson, 1986). The hemolymph pH of cockroaches (*Nauphoetacina*) can be altered when exposed to atmospheric changes; exposure to CO<sub>2</sub> resulted in a substantial drop in blood pH (Matthews & White, 2011). Similarly, exposing *A. domesticus* to CO<sub>2</sub> caused a drop in blood pH and resulted in feeding inhibition, anesthetization, and long-term disruption of certain neuroendocrine functions (Woodring, 1985). Mayfly nymph (*Stenonemafemorum*) whole-body [Na<sup>+</sup>] and [Cl<sup>-</sup>] decreased and mortality rates increased when exposed to acidic conditions of pH 3.5 (Rowe, Berrill, & Hollett, 1988). Because of organic acids' surfactant nature they can more readily permeate the cuticle and cellular membrane than branched or inorganic acids resulting in the acidification of the microbial environment (Legal et al., 1999). An open circulatory system allows the acid's effects to be systemic and rapid. In all trials, 1.36M butanoic acid was 100% lethal to both immature and adult crickets.

The crickets instantly reacted to the spray with violent leg twitching response followed by unresponsive immobilization within seconds-minutes of being sprayed. Specific physical responses varied proportionally with acid concentrations. The presence of SCFAs in *Drosophila melanogaster* was shown to cause a reversible coma-like state associated with altered equilibrium [balance], strong [body] vibrations, and unresponsiveness (Farine et al., 1996). The LC<sub>50</sub> at 24 hours post treatment for immature and mature crickets for propionic, butanoic, and pentanoic acids were calculated (Figures 1, 2, Table 2). Crickets sprayed with distilled water or acidified distilled water (pH = 4.0) were not affected (data not shown). HCL (1.0M, pH=3.6) had a significantly higher survival rate at 24 hours: 87%. The difference between LC<sub>50</sub> for propionic, butanoic and pentanoic acids for immature and mature populations were: 0.28M, 0.13M, and 0.1M, respectively. This study demonstrated the effectiveness of SCFAs as potential insecticides for use against house crickets. Because of their biodegradable properties, SCFAs could be safely used as environmentally friendly, green chemistry, insecticides outdoors without damage soil as would happen with other acids (hydrochloric, sulfuric, etc.). SCFAs are naturally biodegraded by microorganisms in the soil making them a safer alternative to more environmentally-toxic pesticide treatments.

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**Table 1: Cricket Haemolymph pH after Exposure to Short-Chain Fatty Acids**

Treatment	Control	HCl	Propionic acid	Butanoic acid	Pentanoic acid
Conc. (M)	0	1.0	1.5	1.36	0.2
pH	8.24±0.07	8.23±0.11	7.86±0.37 <sup>1</sup>	7.24±0.44 <sup>1</sup>	7.88±0.24 <sup>1</sup>

<sup>1</sup>Significant differences determined by student t-test (P<0.01)

**Table 2: LC-50 (M) for Short-Chain Fatty Acids Sprayed on *A. domesticus***

Stage	Propionic acid	Butanoic acid	Pentanoic acid
Adult	0.82	0.57	0.16
Immature	1.10	0.44	0.26

## Figure Captions

**Fig. 1.** Relationship Between Acid Molarity and Immature Cricket Mortality (LC<sub>50</sub>) 24 Hours Post Treatment.

**Fig. 2.** Relationship Between Acid Molarity and Adult Cricket Mortality (LC<sub>50</sub>) 24 Hours Post Treatment.

Figures

