TOWSON UNIVERSITY
OFFICE OF GRADUATE STUDIES

INSECT OLFACTORY REPELLENTS: IS THERE A GUSTATORY CONTRIBUTION?

by

Jillian Lee Sanford

A thesis

Presented to the faculty of

Towson University

in partial fulfillment

of the requirements for the degree

Master of Science

Department of Biological Sciences

Towson University
Towson, Maryland 21252
December 19, 2013

TOWSON UNIVERSITY
DEPARTMENT OF BIOLOGICAL SCIENCES

REPORT OF RESULTS OF THESIS DEFENSE TO THE GRADUATE PROGRAM DIRECTOR

Name of Student: Jillian Sanford

Title of Thesis: Insect Olfactory Repellents: Is There A Gustatory Contribution?

Date of Defense: 12/2/13

Signatures and Votes of Thesis Committee Members:

	Approve	Disapprove
(Chairperson)	✓	
Member	✓	
Member	✓	
Member		
Member		

Result of thesis defense: Pass ✓ Fail ___
(Unanimous approval is required)

Signature of Thesis Defense Chairperson

Janet V. DeLany Dean Graduate Studies
12/12/13

**Instructions: Fill in the appropriate fields(in bold type), print, and bring to your thesis defense. Tthe Thesis Defense Chair should forward this form to the Biology Graduate Program Director(s) immediately after the defense.*

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Deployed War Fighter Protection Research Program awarded to J. C. D. and grants from TU to J.S. I would like to thank Dr. Vonnie Shields for her unwavering support as my research mentor and her constant guidance and encouragement throughout both my undergraduate and graduate careers. I would also like to thank Dr. Joseph C. Dickens for providing invaluable advice and guidance. I would also like to thank Dr. Jack Shepard for participating in my committee and critically reviewing my thesis. I appreciatively acknowledge Jeff Klupt (Towson University) and Robert Bennet (USDA, Beltsville, MD) for providing technical assistance. A version of chapter one of this thesis has been submitted and published in the journal, *Naturwissenschaften.*

ABSTRACT

INSECT OLFACTORY REPELLENTS: IS THERE A GUSTATORY CONTRIBUTION?

Jillian Lee Sanford

Insect repellents provide protection from biting insects including mosquitoes by affecting the olfactory system. As such, the majority of research carried out to understand the mechanisms behind insect repellency has focused on olfaction. Recent molecular evolutionary studies performed on olfactory receptors demonstrated that these proteins have evolved directly from gustatory receptors. The goal of my study is to determine if repellent compounds interact with the insect gustatory system. I carried out electrophysiological studies on the yellow-fever mosquito *Aedes aegypti* (Diptera: Culicidae) to determine if insect repellents stimulate receptor cells housed within gustatory sense organs (sensilla) located on the mouthparts. Electrophysiological studies show the presence of at least three gustatory receptor neurons (GRNs), one of which is activated by repellent compounds. The information in this study reveals a mechanism by which insect repellents act on the gustatory system, potentially leading to the design of more effective insect repellents.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter One

Chapter Two

CHAPTER ONE

NEUROPHYSIOLOGICAL RESPONSES OF A GUSTATORY RECEPTOR NEURON IN *AEDES AEGYPTI* TO OLFACTORY REPELLENTS

A version of this chapter has been published in the journal, Naturwissenschaften

INTRODUCTION

The use of insect repellents has contributed to decreasing contact between insect disease vectors and their host. The compound N,N-diethyl-meta-toluamide, referred to as DEET, is the most widely used chemical in commercially available repellents (Brown and Hebert, 1997). In recent years, new synthetic repellents including amides, piperidines, and diols have been discovered (Paluch et al., 2010). DEET, 3-[N-Butyl-N-acetyl]-aminopropionic acid ethyl ester (IR3535), and 2-(2-hydroxyethyl)-1-piperidine carboxylic acid 1-methylpropyl ester (picaridin) have all been shown to elicit repellent behavior in insects within the family Diptera (Chauhan et al., 2005; Klun et al., 2006; Licciardi et al., 2006; Syed et al., 2011). The modes of action behind the repellency of these compounds remain unclear. Both Dethier (1960) and Davis (1985) state that a compound can elicit more than one behavior and cause insects to orient away from potential hosts and inhibit it from feeding on a natural food sources. DEET and picaridin elicit feeding deterrent behavior in dipterans (Bar-Zeev and Schmidt, 1959; Klun et al., 2006). The ability of these repellents to elicit a feeding behavioral response in insects, suggests that these olfactory compounds elicit responses in the gustatory receptors. However, limited information is available on the interactions between insect repellents and the gustatory system of insects. Dipterans have specialized sensory organs (sensilla) located on various regions of the body, including the antennae and maxillary palps (McIver and Siemicki, 1984; Dickens et al., 1988; de Bruyne et al. 1999; Pitts and Zwiebel, 2006; Hill et al., 2009). The olfactory sensilla house olfactory receptor neurons (ORNs) responsible for detecting ecologically relevant compounds (Syed and Leal, 2007; Ghaninia et al., 2007; Syed and Leal, 2008; Ghaninia et al., 2008; Hill et al., 2009; Cook

et al., 2011). Many dipteran species have an ORN sensitive to repellent compounds, which may mediate, at least in part, aversive behavior (Boeckh et al., 1996; Ditzen et al., 2008, Syed and Leal, 2008; Bohbot and Dickens, 2010; Kwon et al., 2010; Bohbot and Dickens, 2011; Bohbot and Dickens, 2012). These studies provide strong evidence for olfaction mediating aversive behavior elicited by insect repellents.

Recent genetic studies have demonstrated a close relationship between smell and taste (Scott et al., 2001; Galindo and Smith, 2001; Robertson et al., 2003). In dipterans, there is mounting evidence that olfactory (ORs) and gustatory receptors (GRs) share a common gene origin (Scott et al., 2001; Robertson et al., 2003). These GRs are expressed in cell membranes of gustatory receptor neurons (GRNs) housed within sensilla and are specific for various tastants in the environment (Isono and Morita, 2010). Gustatory sensilla are located on multiple regions of the body including the proboscis (mouthparts), tarsi (legs), wings and ovipositor of females (Isono and Morita, 2010). While, the GRs in insects have putative seven-transmembrane domain motifs, as in mammals, interestingly, recent studies have shown insect taste transduction may not use G-coupled second messenger signaling cascades, but may occur through ligand-gated ion channels specific for certain compounds (i.e., sugar) (Kijima et al., 1988; Murakami and Kijima, 2000; Sato et al., 2011). Recently, Lee et al. (2010) demonstrated DEET activates GRNs housed in sensilla located on the labellum of the mouthparts in the vinegar fly *Drosophila melanogaster.* and identified GRs responsible for its detection.

The goal of my project was to determine if GRNs in the labellar lobes of another dipteran, the yellow-fever mosquito *Ae. aegypti* respond to the repellent compounds DEET, IR3535, and picaridin. *Ae. aegypti* vector numerous viral diseases including,

yellow fever, dengue, and chikungunya (National Center for Emerging and Zoonotic Infectious Diseases). *Ae. aegypti* is capable of surviving and reproducing in urban environments and its eggs are capable of withstanding long periods of desiccation. These characteristics contribute to making it a particularly dangerous disease vector. Sensilla located on the proboscis of *A. aegypti* are prime candidates for mediating the detection of repellent compounds such as DEET, IR3535, and picaridin. The results of this study further our understanding of the modes of action of repellent compounds and could contribute to the development of more potent and effective repellents.

MATERIALS AND METHODS

Animals

Ae. aegypti (Gainesville strain) were reared from eggs (The Center for Medical and Veterinary Entomology, USDA, FL, USA) and maintained at 25ºC, 20-40% relative humidity under a 12 light:12 dark photoperiod regimen. Larvae were fed ground Tetramin® tropical (Tetra) fish food. Adults were maintained in an incubator (Percival) at 27ºC at 70% relative humidity and same photoperiod regimen as the larvae. Adult mosquitoes were fed a 10% sucrose solution *ad libetum*. Female mosquitoes, 5-10 days post emergence, were used for all experiments as this sex is responsible for biting the host and spreading human dengue fever.

Electrophysiology

A modified tip recording method (Hodgson et al., 1955) was used that involved immobilizing the insect and placing a tungsten ground electrode into the insect's body. The recording electrode was a glass capillary with a tip fashioned to a diameter large

enough to fit over the tip of a single sensillum. This electrode was filled with an electrolyte solution (10mM NaCl) in addition to the test compound. Each recording of electrical activity of neurons within a sensillum lasted for 10s. A minimum of 3 min was offered between stimulations to allow the sensillum to return to its resting state.

Electrical activity of neurons housed with labellar sensilla was determined for 0.1M sodium chloride (control compound), sucrose (a feeding stimulant), and quinine (a feeding deterrent; Ignell et al., 2010). Action potentials of GRNs were characterized based on amplitude and shape.

Sensilla in *Ae. aegypti* were be stimulated each with 0.08% (%v/v) DEET, IR3535, and picaridin and were tested with increasing concentrations of sodium chloride, sucrose, and quinine spanning four to five logarithmic steps (e.g., 0.001mM, 0.01mM, etc.) and DEET (0.00008%, 0.0008%, etc.). The selection of specific concentrations was in keeping with results of a previous study in the vinegar fly, *Drosophila melanogaster* (Lee et al., 2010). For all experiments, the total number of action potentials produced were quantified for a 1s 50ms following initial contact of the recording electrode.

RESULTS

Electrophysiological responses recorded from labellar sensilla in *Ae. aegypti* revealed three different classes of action potentials based on amplitude and shape, likely representing activity of three gustatory receptor neurons (GRNs). The GRN activated by salt produced a large amplitude action potential (Fig 1). The GRN activated by sugar produced an action potential with amplitude similar to that of salt, with a negative slope less precipitous than that of the GRN activated by salt (Fig 1). The third GRN, activated by the feeding deterrent, quinine, produced a much smaller amplitude action potential

than either the salt or sugar GRN (Fig 1). Dose-response curves for three taste stimuli revealed a detection threshold of 10mM for sodium chloride and 1mM, for both sugar and quinine (Repeated Measures ANOVA; post-hoc Tukey-Kramer analysis; $\alpha = 0.05$; n = 6 for NaCl, n = 5 for all other compounds) (Fig 2.A-C).

Stimulations of labellar sensilla in *A. aegypti* with the repellents DEET, IR3535, and picaridin all elicited small amplitude action potentials (Fig 4). Similarly, a mixture of 1mM quinine and 0.08% DEET or 0.08% DEET, IR3535, and picaridin activated a single GRN producing a small amplitude action potential (DEET, 27.8 ± 5.6, IR3535, 28.3 ± 6.3, picaridin, (33.5 ± 8.0) and elicited significantly more small amplitude action potentials than control solution (5.7 ± 1.7) (repeated measures ANOVA; post-hoc Tukey Kramer analysis; $\alpha = 0.05$; n = 6) (Figs. 3, 5). Dose-response curves for DEET revealed a detection threshold of 0.08% (repeated measures ANOVA; post-hoc Tukey Kramer analysis; $\alpha = 0.05$; n = 5) (Fig 2.D).

DISCUSSION

Hodgson and Roeder (1956) performed the first electrophysiological examination of the labellar gustatory sensilla in two dipteran species, *Phromia regina* Meigen and *Sarcophaga bullata* Parker and found the sensilla were sensitive to both salts (i.e., NaCl, KCl, and NaBr) and sugars (i.e, sucrose, d-glucose, and d-arabinose). Subsequent studies performed on chemosensilla located on the labella of other dipteran species showed sensitivities to other compounds including deterrents, water, and a range of other salts and sugars (Gothilf et al., 1971; Hiroi et al., 2002; Liscia et al., 1995; Liscia et al., 1998; Liscia and Solari, 2000; Maes and Vedder, 1978; Pappas and Larsen, 1976; Stoffolano et.

al. 1990). In this study, labellar gustatory sensilla of female *A. aegypti* responded to a salt (NaCl), sugar (sucrose), and a feeding deterrent [i.e., quinine (Ignell et al., 2010)]). Lee and Craig (2009) demonstrated that the labellar sensilla on the proboscis of *Ae. aegypti* are innervated by 3-5 neurons, which is congruent with the findings in this paper.

We found the deterrent GRN in *Ae. aegypti* was stimulated by three synthetic insect repellents: DEET, IR3535, and picaridin. The deterrent GRN responded in a dose-response manner when exposed to increasing concentrations of the repellent DEET. Concentration-dependent tests performed with quinine and DEET had detection thresholds at similar concentrations (1mM and 4.21mM, respectively). This suggests the GRN has a similar sensitivity level to both the feeding deterrent and synthetic repellent chemical.

The activation of a single GRN in response to a feeding deterrent and three synthetic repellent chemicals indicates a role for gustation in the repellent response observed in insects exposed to these chemicals. In *D. melanogaster*, Lee et al. (2010) found that feeding on higher concentrations of sugar was deterred by low concentrations of DEET. In the same study, deletion of a gustatory receptor gene, necessary for DEET sensitivity, resulted in the loss of sensitivity to natural feeding deterrents, such as strychnine. An early study performed by Bar-Zeev et al. (1959) observed that addition of DEET to blood inhibited feeding by female *A. aegypti*. Additionally, Chauhan et al. (2005) found that application of DEET to skin caused a significant decrease in biting by female *Ae. aegypti*. This feeding deterrence may be mediated by a GRN sensitive to repellent chemicals.

While this study suggests a role for taste in mediating insect repellency, more research is necessary to elucidate the molecular mechanisms. Knowledge of the underlying mechanisms of mediating insect repellency will facilitate design improved chemical repellents and improve strategies for their use in the protection of humans and animal hosts.

FIGURE HEADINGS

Figure 1: Representative traces from a labellar sensillum in *Ae. aegypti* to sodium chloride, sucrose and quinine. Traces are 400ms. The GRN activated by sodium chloride produced large amplitude action potentials. Sucrose stimulated action potentials similar in amplitude but differing in shape to that of NaCl. Quinine and DEET elicited action potentials of smaller amplitude compared to those elicited by NaCl and sucrose.

Figure 2: Dose-response curves constructed from responses of neurons associated with sensilla on the labella of *Ae. aegypti* females to serial dilutions of experimental stimuli. The number of action potentials was quantified over 1s to increasing concentrations of A) sodium chloride, B) sucrose, C) quinine, and D) DEET. The threshold concentration at which a significant increase in spiking occurred was indicated by an asterisk.

Figure 3: Representative traces of electrical activity of neurons associated with labellar sensillum in *Ae. aegypti* to 0.08% DEET, 1mM quinine, and a mixture of 0.08% DEET and 1mM quinine. Examples of the action potential produced when the repellent GRN is activated are shown. Quinine and DEET activate the GRN that produces an action potential with small amplitude, short positive phase, and long negative phase. This is confirmed by the stimulation with the mixture.

Figure 4: Representative traces of electrical activity of neurons associated with a labellar sensillum of *Ae. aegypti* to 0.08% (%v/v) DEET, picaridin, and IR3535. Traces are 400ms. Examples of the action potential produced when the repellent GRN is activated are shown. All three repellents activate the GRN that produced an action potential with small amplitude.

Figure 5: Total number of action potentials produced from 0.05-1.05s after initial stimulation of sensilla in *Ae. aegypti* with 0.08% (%v/v) DEET, IR3535, and picaridin. Asterisks indicate a significant increase in the firing rate of the deterrent cell when compared to that of the control (i.e., NaCl).

FIGURES

Figure 1:

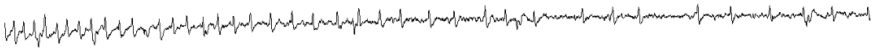
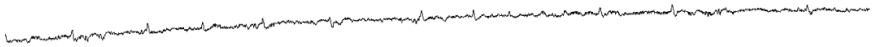
Compound	Response in a Labellar Sensillum	Shape
1000mM NaCl		
100mM Sucrose		
1mM Quinine		
	.5 mV 50 ms	

Figure 2:

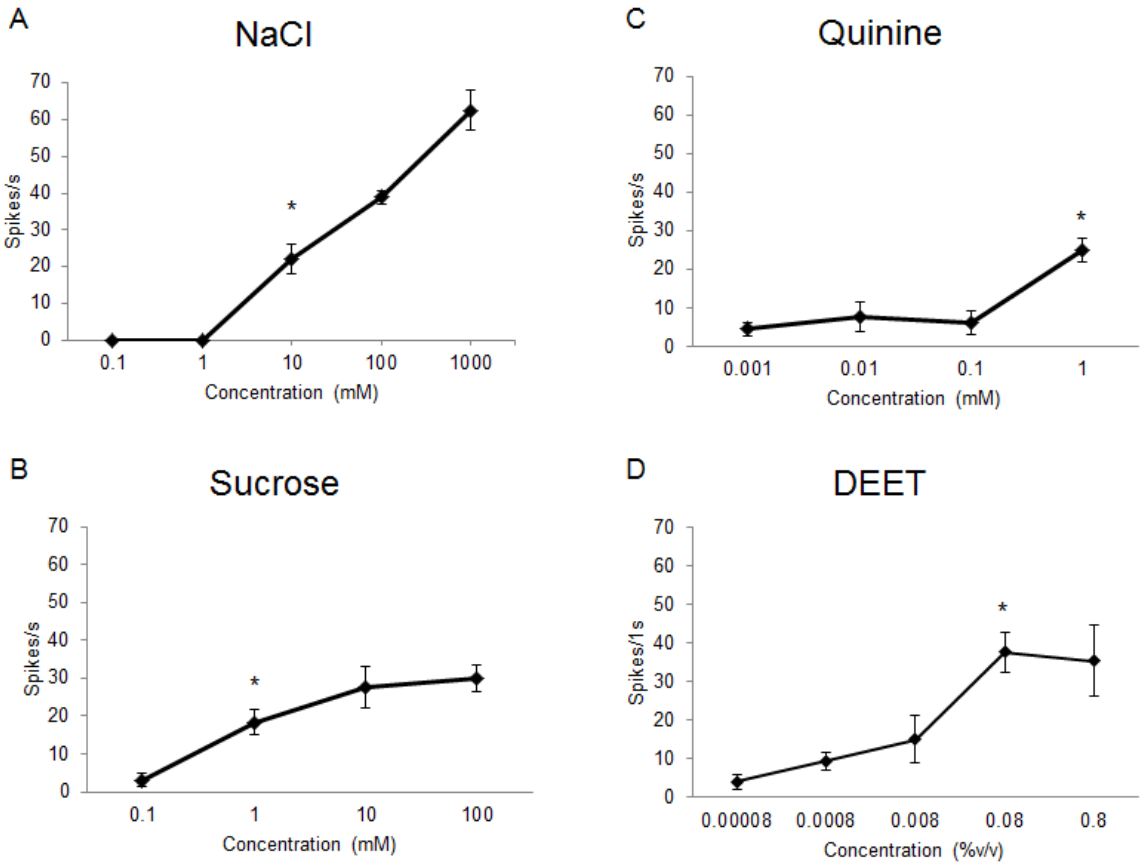

A
NaCl
Spikes/s
70
60
50
40
30
20
10
0
0.1
1
10
100
1000
Concentration (mM)
*
C
Quinine
Spikes/s
0.001
0.01
0.1
1
Concentration (mM)
*
B
Sucrose
Spikes/s
0.1
1
10
100
Concentration (mM)
*
D
DEET
Spikes/1s
0.00008
0.0008
0.008
0.08
0.8
Concentration (%v/v)
*

Figure 3:

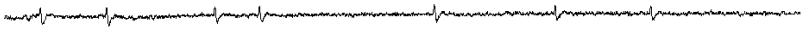
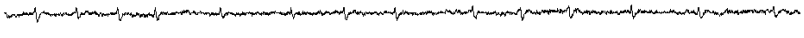
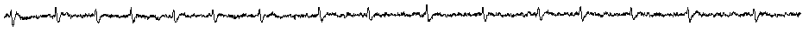
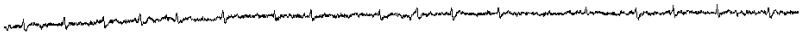
Compounds	Responses in Labellar Sensilla	Shape
0.08%DEET		
1mM Quinine		
0.08% DEET + 1mM Quinine		
0.08% DEET + 0.08% IR3535 + 0.08% Picaridin		
	.5 mV 50 ms	

Figure 4:

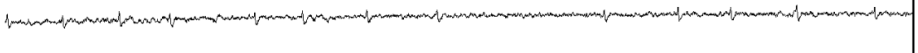
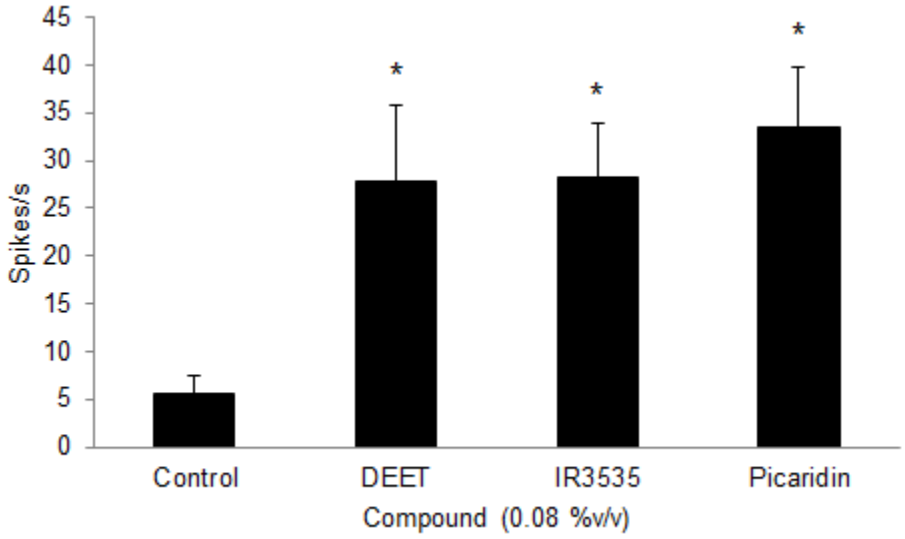
Compound	Responses in a Labellar Sensillum	Shape
0.08% DEET		
0.08% Picaridin		
0.08% IR3535		
	.5 mV 50 ms	

Figure 5:


45
40
35
30
25
20
15
10
5
0
Spikes/s
*
*
*
Control
DEET
IR3535
Picaridin
Compound (0.08 %v/v)

CHAPTER TWO

NEUROPHYSIOLOGICAL RESPONSES OF THE DETERRENT-SENSITIVE GUSTATORY RECEPTOR NEURON IN *LYMANTRIA DISPAR* TO OLFACTORY REPELLENTS

ABSTRACT

Interactions between insect repellents and the olfactory system have been widely studied. To date, little is known about the interactions of these repellents with the gustatory system. While recent work indicates a direct association between insect repellents and the adult gustatory system, evidence lacks showing a link between the interaction of repellent chemicals and the larval taste system. Gypsy moth larvae have a deterrent-sensitive gustatory receptor neuron (GRN) that responds to feeding deterrents. I tested whether the GRN responded to three olfactory repellents, namely DEET, IR3535, and picaridin. I found that all three repellents stimulated the same deterrent-sensitive GRN, which responded phasic-tonically and in a dose-dependent manner. While there has been a behavioral study examining the effect of repellents on larval feeding in *Anopholes gambiae* (e.g. Xia et al. 2008), this is the first electrophysiological study, to our knowledge, that demonstrates gustation contributes to the perception of repellent chemicals.

INTRODUCTION

N, N-diethyl-3-m-toluamide (DEET) is a popular insect repellent that is used in many commercial bug sprays, including "Repel" and "Off" (Brown and Hebert, 1997; Katz et al., 2008). DEET is of particular importance because it is capable of repelling numerous insect vectors of harmful diseases (Brown and Hebert, 1997). Over the last twenty years, great strides have been made to elucidate the mechanisms of insect repellency as it pertains to olfaction (e.g., Vosshall and Stocker, 2010; Dickens and Bohbot, 2013). While it is known that olfaction plays a major role in mediating the repellency of DEET and other insect repellents, recent research on two selected adult dipteran species has clearly demonstrated that repellents may also act through their gustatory system (Lee et al. 2010; Sanford et al. 2013). These authors demonstrated that two adult dipteran species, the vinegar fly *Drosophila melanogaster* (Lee et al. 2010) and the yellow-fever mosquito *Aedes aegypti* (Sanford et al. 2013), have a gustatory receptor neuron (GRN) housed within the labellar sensilla sensitive to DEET (*D. melanogaster*) and three other common insect repellents (*Ae. aegypti*). Lee et al. (2010) also showed that DEET deterred feeding. Another study demonstrated behavioral responses of larvae of the malaria vector mosquito *Anopheles gambiae* (Xia et al. 2008), however, it is not known if DEET and other insect repellents are detected by contact chemosensilla in larvae of other species, especially those of agricultural importance. If so, this could lead to the development of novel insect antifeedants against agriculturally destructive larval species.

The chemosensilla of gypsy moth *Lymantria dispar* (Lepidoptera: Erebidae) larvae are located on various sense organs including the antennae, epipharynx, galeae,

and maxillary palps (Schoonhoven and Dethier, 1966). The maxillary palps each bear two pairs of gustatory sensilla, the lateral and medial styloconic sensilla, that have been well characterized (Shields, 2009; Martin and Shields 2012a,b). These sensilla in this species and other lepidopteran larvae are innervated by 4 gustatory receptor neurons (GRNs) that respond to different taste modalities (Shields 2009; Martin and Shields 2012a; Martin and Shields 2012b)., A deterrent GRN located in the medial styloconic sensillum, is activated by secondary plant compounds such as alkaloids (Martin and Shields, 2012b) which deter feeding (Shields et al; 2006; 2008). The deterrent GRN in gypsy moth larvae suggest it as a candidate to investigate whether or not repellents, such as DEET, impact the gustatory system of lepidopteran larvae.

MATERIALS AND METHODS

Insects

Lymantria dispar eggs (New Jersey strain) were obtained from USDA, APHIS, Otis Air National Guard Base in Falmouth, Massachusettes, USA. Caterpillars were reared on a high wheat germ-based artificial diet (Bio-Serv, Frenchtown, New Jersey; MP Biomedicals, Solon, Ohio, USA) and maintained at 27ºC ± 2ºC and 60% relative humidity in a 12-h light/12-h dark photoperiod regimen. Fifth instar larvae, 12-18 h postmolt, and 24-h food-deprived were used in all experiments. The larvae were naïve to the test compounds prior to experimentation. Fifth instar larvae were used as this stage is the most ecologically destructive (Leonard 1974, 1984; Lance and Barbosa 1982).

Electrophysiology

Electrical responses from taste receptor cells within the medial styloconic sensilla were recorded using an extra-cellular tip-recording method (after Hodgson et al. 1955;

Shields and Mitchell 1995a) (Fig. 1). The recording procedure involved mounting the head of the caterpillar on a saline-filled ground electrode containing a silver wire. A stimulating electrode was then placed over the tip of a styloconic sensillum for recordings. A minimum of 3 min was allowed between successive stimulations. Selection of right or left sensilla was randomized. Presentations of stimulating solutions were given in a randomized order to prevent bias, except for concentration-response experiments in which solutions were given in order of increasing molar concentration. All recordings were made between 0900 and 1700 h during light of the photoperiod.

Electrical activity was recorded from individual styloconic sensilla, amplified 10x and passed through a bandpass filter set at 100-1,200 Hz (Syntech, Hilversum, the Netherlands). Recordings were digitized by a 16-bit analog-to-digital interface (IDAC-4 Syntech) and analyzed off-line with Autospike software (version 3.8) (Syntech). For each electrophysiological recording, a single sensillum was stimulated for a total of 10 s. Only the action potentials generated 50 ms after contact of the sensillum by the stimulating electrode were analyzed using the Autospike software (Syntech, Hilversum, the Netherlands).

Taste Stimuli

Stimulus compounds were dissolved in aqueous 30 mM potassium chloride (control solution) (KCl) (Fisher Scientific, Fair Lawn, New Jersey) (Martin and Shields, 2012a,b) in distilled water to enhance the electrical conduction of the recording electrode to improve the signal-to-noise ratio (Martin and Shields 2012b). This solution was also used to fill the indifferent electrode. All solutions were tested at room temperature (20ºC ± 2ºC) and comprised the following: the repellents DEET (Sigma-Aldrich Co., St. Louis,

MO, USA , IR3535 (Merck KGaA, Darmstadt, GE), and picaridin (LANXESS, Pittsburg, PA, USA). The repellents were first dissolved in 10% ethanol and 30mM KCl was added to give a final concentration of 10mM (repellent stock solution). At this concentration, ethanol had no discernible effect on the electrical activity of the taste receptor cells (Martin and Shields 2012b). The majority of tests were carried out with dilutions of this repellent stock solution, in which ethanol comprised 0.1% or less.

Data Analysis

Each recording was visualized using the "Amplitude Histogram View" feature in Autospike, which allows for identification and assignment of spike amplitudes to different taste receptor cells. For experiments examining the activation of the deterrent GRN by the repellents, a GLM ANOVA ($\alpha = 0.05$) to test the null hypothesis that there is no difference in the response to the repellents compared to that of the control. The repellent solution was the fixed variable and the firing frequency was the response variable. A Tukey-Kramer multiple comparison test was used to compare responses of the deterrent GRN to the repellents.

Examination of dose response experiments was done using a repeated measure analysis of variance (ANOVA) ($\alpha = 0.05$). This test was used to test the null hypothesis that there is no difference in the response of the deterrent GRN among all concentrations of each repellent compared with that of the control. Each sensillum was the subject variable, the concentration was the within-factor/repeated variable, and the firing frequency was the response variable. A Tukey-Kramer multiple comparison test was used to compare responses of the deterrent GRN to various concentration of each repellent.

A repeated measure analysis of variance (ANOVA) ($\alpha = 0.05$) was used to determine the temporal dynamics of response curves, represented by the mean number of spikes sampled in 100-ms time bins across 3,000ms. Each sensillum was the subject variable, time intervals were the within-factor/repeated variable, and the firing frequency was the response variable. A Tukey-Kramer multiple comparison test was used to compare responses of each GRN to determine when a transient (i.e., phasic) pattern of firing changed to that of a sustained (i.e., tonic) pattern of firing. Data was analyzed using Excel (Microsoft Corp. Redmond, WA, USA) and NCSS (Kaysville, UT, USA).

RESULTS

Responses of deterrent-sensitive GRN to olfactory repellents

A single cell fired in response to all three repellent chemicals tested (i.e., DEET, IR3535, and picaridin) (Fig 2.B-D). This was verified by stimulations of the medial styloconic sensillum with a mixture of all three repellent chemicals, where only the response of a large-amplitude producing cell was observed (Figure 2.E). All three repellents elicited response of this large-amplitude producing cell, which was also similar in height to that elicited by 30mM KCl (Fig 2.A). Previous electrophysiological recordings performed on the medial styloconic sensillum characterized this large-amplitude producing cell, as the "deterrent-sensitive cell" (Martin and Shields, 2012b), responding to natural deterrent compounds, such as caffeine, nicotine, strychnine, and aristolochic acid. To further confirm that the three olfactory repellent chemicals activated the same deterrent-sensitive GRN, the medial styloconic sensillum was stimulated with a mixture of 10mM each of DEET, IR3535, picaridin, and caffeine. Only

one type of spike, with the characteristics of those produced by the deterrent-sensitive cell (i.e., large-amplitude spike), was observed after stimulation with the mixture (Fig 2.F). To further confirm activation of the deterrent-sensitive cell by these three repellent chemicals, the total number of spikes produced 0.05-1.05s after stimulation with each of the repellents were quantified and compared with that of the control solution (i.e., 30 mM KCl). Each repellent compound elicited significantly more spikes compared with that of the control (2.G) (GLM ANOVA; post-hoc Tukey-Kramer analysis; $\alpha < 0.05$; $n = 8$ sensilla). Additionally, IR3535 elicited significantly more spikes than either DEET or picaridin (2.G) (GLM ANOVA; post-hoc Tukey-Kramer analysis; $\alpha < 0.05$; $n = 8$ sensilla).

Concentration-response characteristics of deterrent-sensitive GRN

Dose response curves were constructed to characterize GRN activity in response to serial dilutions of the repellents: DEET, IR3535 and picaridin. A typical dose response to one of the repellents, IR3535, is shown in Fig. 3. The deterrent-sensitive GRN responded in a dose-dependent manner to increasing concentrations of all repellents (Fig 4.A-C). This cell was equally sensitive to IR3535 and picaridin with a threshold concentration of 1mM (Fig 4.A and B) (repeated measures ANOVA; post-hoc Tukey-Kramer analysis; $\alpha < 0.05$; $n = 5$-6 sensilla). The threshold concentration for DEET was however, 1 decade higher at 10mM) (Fig 4.C) (repeated measures ANOVA; post-hoc Tukey-Kramer analysis; $\alpha < 0.05$; $n = 5$-6 sensilla). Higher concentrations of DEET were not tested because of the inability of the chemical to solubilize in the control solution. The response of the deterrent-sensitive cell to IR3535 and DEET did not plateau;

however, the response of the deterrent-sensitive cell to picaridin appeared to plateau at 10mM (Fig 4.A- C).

Temporal response characteristics of the deterrent-sensitive GRN

In order to quantify the temporal dynamics of response to the repellents, the average number of spikes that occurred in successive 100ms time intervals across the first 3s after stimulation was determined. All three repellent chemicals elicited a phasic-tonic response from the deterrent-sensitive cell (Fig. 5). IR3535 elicited the most robust firing frequency (15 ± 1.4 spikes) within the first 100ms after stimulation, while picaridin (9.7 ± .9 spikes) and DEET (7.2 ± .7 spikes) elicited fewer spikes within the same time period (Fig 5.C). The deterrent-sensitive GRN displayed a similar tonic pattern of firing to DEET, IR3535 (1300ms after initial stimulation) (Fig 5.A and C), and picardin (1200ms after initial stimulation) (Fig 5.B) (repeated measures ANOVA; post-hoc Tukey-Kramer analysis; $\alpha < 0.05$; n = 13-16 sensilla).

DISCUSSION

Although the interactions between insect repellents and the olfactory system have been widely studied, relatively little is known about their interactions with the gustatory system. While, recent research has demonstrated a direct link between insect olfactory repellents and the adult gustatory system (Lee et al. 2010 and Sanford et al. 2013), nothing is known about how these repellents interact with the larval taste system, save for a behavioral study involving mosquito larvae (Xia et al. 2008). Here we demonstrate, for the first time, using a lepidopteran larva as a model system, that insect repellents are detected by a specific gustatory neuron in the larva of the *L. dispar*.

Repellents and activation of the deterrent cell

Gustatory receptor neurons of polyphagous caterpillars are capable of detecting multiple chemical classes (Bernays 2000; Glendenning 2006). One class of chemicals, deterrents, activates a specialized deterrent-sensitive neuron housed within gustatory sensilla in many lepidopterans (Descoins and Marion-Poll, 1999; Rharrabe et al., 2011; van Loon and Schoonhoven, 1999; Schoonhoven and Lin-er, 1994; Zhou et al., 2010). Martin and Shields (2012b) characterized the deterrent-sensitive neuron in the medial styloconic sensillum on the galea of *L. dispar*. This deterrent-sensitive neuron was sensitive to the alkaloids, aristolochic acid, caffeine, nicotine, and sailicin. In the present study, we found that three olfactory repellent chemicals (DEET, IR3535, and picaridin) activated the same large-amplitude deterrent-sensitive cell. Stimulation with a mixture of caffeine and the three repellents compounds showed activity of a single cell, consistent with selective activation of the large-amplitude deterrent-sensitive cell. These results are consistent with findings in *A. aegypti*, where a single GRN was activated by mixtures of the repellent DEET and a feeding deterrent (Sanford et al. 2013). In another behavioral study, DEET, IR3535, and picaridin were shown to deter feeding in *L. dispar* larvae (in preparation).

Characterization of responses of the deterrent sensitive cell to DEET, IR3535, and picaridin

Dose response curves to the three repellents showed a detection threshold concentration of 1mM for IR3535 and picaridin, while a threshold of 10mM was found for DEET. These findings are in keeping with previous electrophysiological studies performed on *A. aegypti* with DEET which had a threshold of 4.2mM to DEET (Sanford

et al., 2013). These compounds all elicited a phasic-tonic pattern of neuronal activity similar to the firing pattern described by Martin and Shields, (2012b) for the alkaloids caffeine, nicotine, and strychnine. These similar temporal firing patterns may represent activation of the same excitatory transduction pathway by the deterrent-sensitive GRN (Glendinning and Hills 1997; Martin and Shields 2012b). The action of all three repellent chemicals may be processed by the central nervous system in a similar fashion to alkaloids (see Shields et al., 2006 and Shields et al., 2008). It is interesting to note the phasic firing response of the deterrent GRN became tonic at nearly identical times for DEET, IR3535, and picaridin (1300ms, 1300ms, and 1200ms, respectively).

Chemical basis for deterrence caused by repellents

DEET, IR3535, and picaridin share an amide (–NC(=O)) moiety, the putative group responsible for mediating repellency in DEET and picaridin (Natarajan et al. 2005). Amides are common plant defense mechanisms against herbivorous insects that increase mortality, deter feeding and cause decreased pupal weights (Dyer et al. 2003; Richard et al. 2010). The fact that picaridin elicited a more robust response than DEET in the deterrent sensitive neuron might be explained by the presence of a piperidine moiety in picaridin. Natarajan et al. (2005) suggested that the presence of the piperidine ring was positioned the amide moiety in a configuration that contributed its repellent effect. The piperidine moiety found in some alkaloids contributes to feeding deterrency in some lepidopterans (Park et al. 2002; Tavares et al. 2011). At a given concentration, we found that IR3535 elicited a more robust response compared with DEET or picaridin. We suggest that this increased firing of the deterrent-sensitive cell may be attributed to the presence of an additional ester moiety absent in DEET or picaridin.

Interestingly, the alkaloids caffeine and strychnine, which have been shown to elicit feeding deterrence in *L. dispar*, contain an amide moiety and were also found to elicit robutst responses in *L. dispar* larvae (Shields et al. 2006; Martin and Shields, 2012b). Since the amide moiety exists in synthetic repellents and naturally occurring deterrents, it is a possible candidate for suggesting the mechanism by which the response of the deterrent GRN is mediated.

The results of our study clearly show that the larval gustatory system of *L. dispar* is sensitive to the insect repellents, DEET, IR3535, and picaridin. These compounds elicit action potentials from a GRN located in the medial styloconic sensilla of this species. While these repellents have been shown to interact with the gustatory system in adults, this study clearly demonstrates the contribution of the sense of taste in larvae in mediating the effects of these repellent compounds. They may also be possible candidates to suggest in the agricultural arena as antifeedants against ecologically destructive larval species.

FIGURE LEGENDS

Figure 1: Diagram of the tip recording technique used for this study. The stimulating electrode is filled with an electrolyte solution (30mM KCl in *L. dispar*) and a repellent compound is placed over the tip of the sensillum. Activation of the GRNs in the sensillum is observed in the form of action potential on a computer. Adapted from Shields and Martin (2011).

Figure 2: Representative traces of responses elicited from gustatory receptor neurons in the medial styloconic sensilla in *L. dispar* to: (a) 30mM KCl, (b) 10mM DEET, (c) 10 mM IR3535, (d) 10mM Picaridin, (e) a mixture of 10 mM DEET, IR3535 and picaridin, and (f) a mixture of 10mM caffeine, DEET, IR3535, and picaridin. Up-arrowheads represent the response of the large-amplitude deterrent-sensitive cell and bars represent the response of the small-amplitude KCl-sensitive cell. (g) Total number of spikes produced 0.05-1.05 s after initial stimulation of the medial styloconic sensilla by the deterrent-sensitive cell to 10 mM DEET, IR3535, and picaridin compared to the control (30mM KCl in 10% ethanol). Different letters represent significant differences between groups. Error bars represent standard errors.

Figure 3: Representative traces of responses elicited from the deterrent-sensitive gustatory receptor neuron contained in the medial styloconic sensilla in *L. dispar* to increasing concentrations of IR3535. Up-arrowheads represent the response of the large-amplitude deterrent-sensitive cell.

Figure 4: Dose-response curves using insect repellent chemicals. Total number of spikes produced by the deterrent-sensitive cell 0.05-1.05s after initial stimulation with

increasing concentrations of a) IR3535, (b) picaridin, and (c) DEET. For each graph, the concentration at which a significant increase in spiking occurred is indicated by an asterisk.

Figure 5: Response curves representing the temporal dynamics of the deterrent-sensitive cell to 10mM concentrations of three repellents: (a) IR3535, (b) picaridin, and (c) DEET. All repellents elicited a phasic-tonic firing pattern from the deterrent-sensitive cell. Asterisks mark the beginning of the tonic firing pattern for each repellent tested. Activity of the deterrent-sensitive cell peaked within the first 100-200ms in response to each repellent. Firing of the cell gradually decreased over the next 800 ms for picaridin and 1000 ms for DEET and IR3535. Firing of the deterrent-sensitive cell became tonic after 1200 ms for picaridin and 1300 ms for DEET and IR3535.

Figure 1

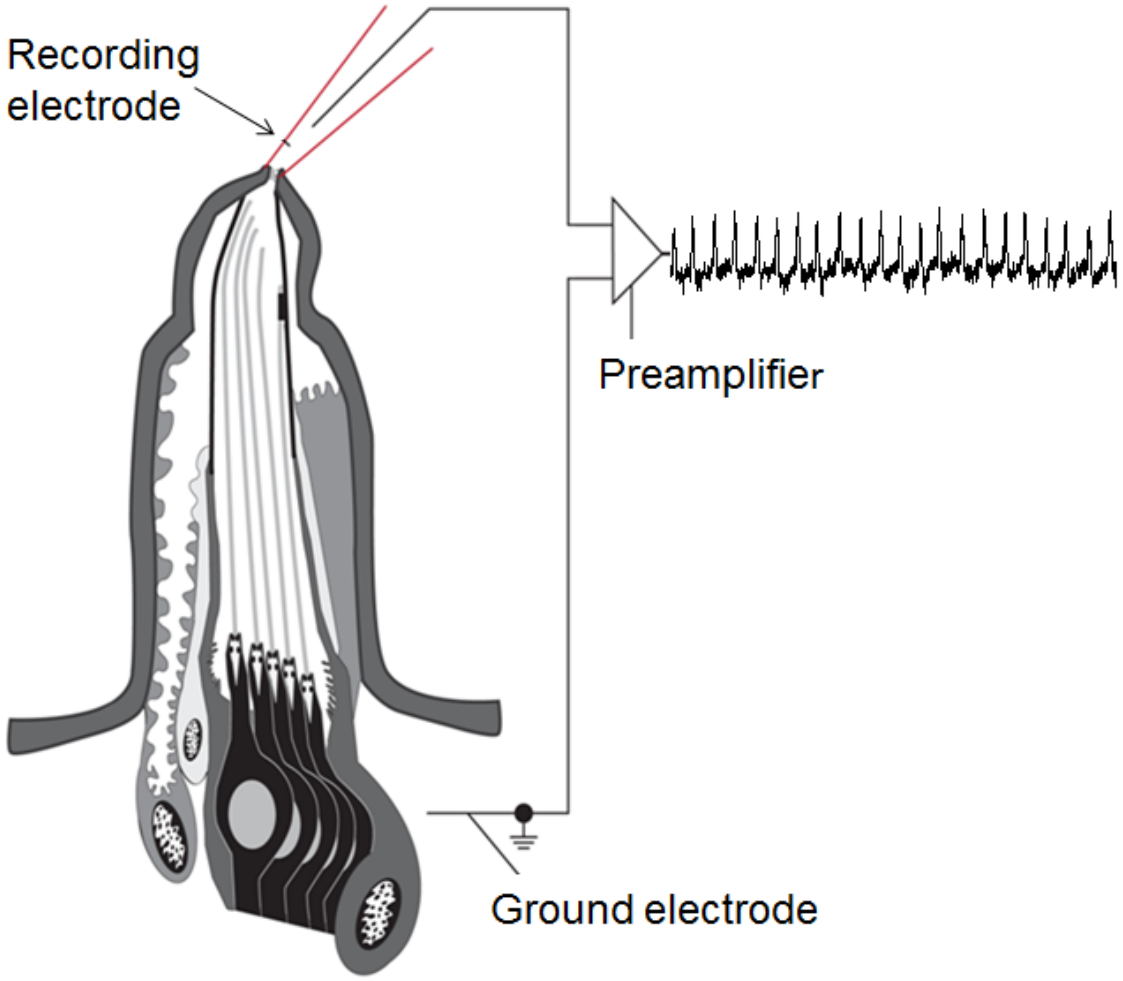


Figure 2

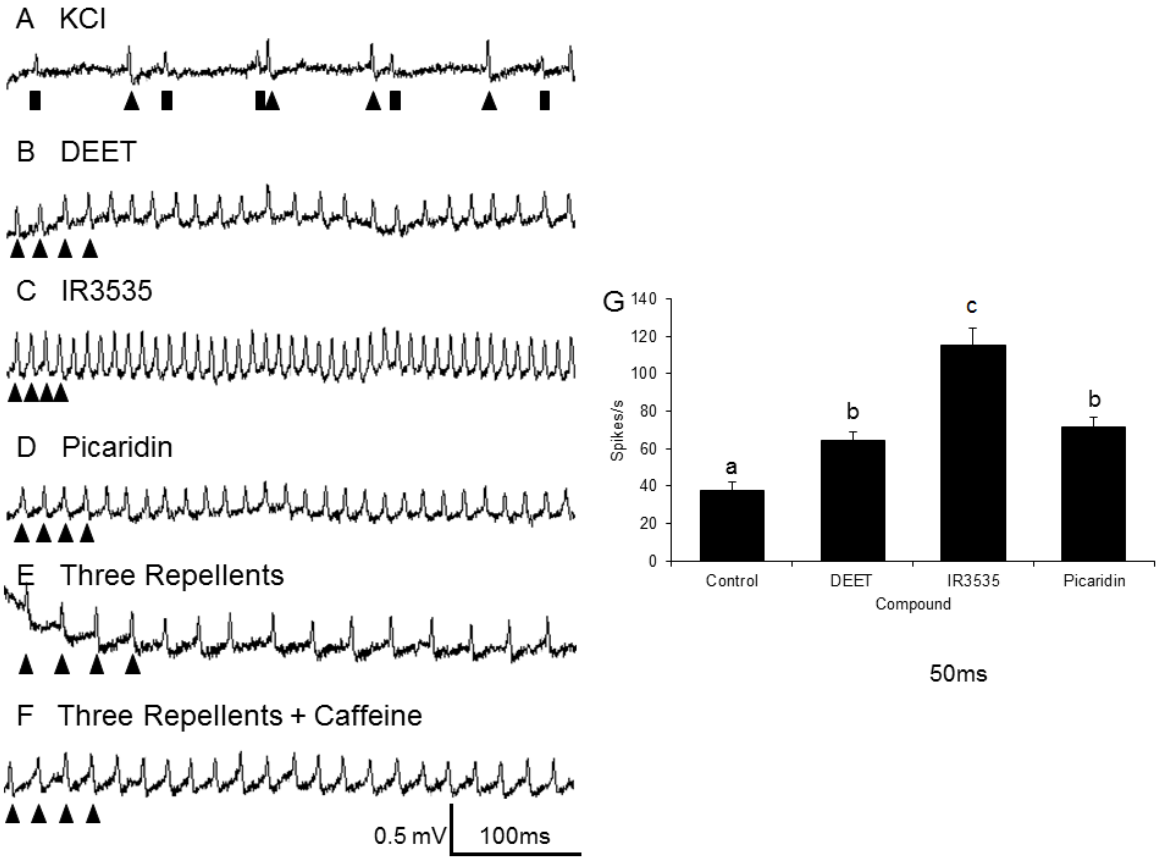

A KCl
B DEET
C IR3535
D Picaridin
E Three Repellents
F Three Repellents + Caffeine
0.5 mV
100ms
G
Spikes/s
140
120
100
80
60
40
20
0
a
b
c
b
Control
DEET
IR3535
Picaridin
Compound
50ms

Figure 3

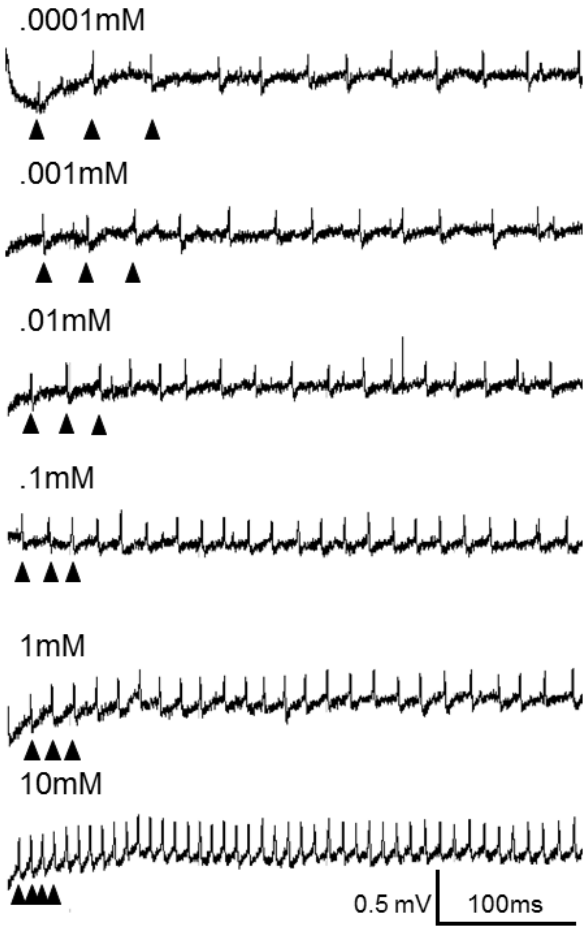


Figure 4

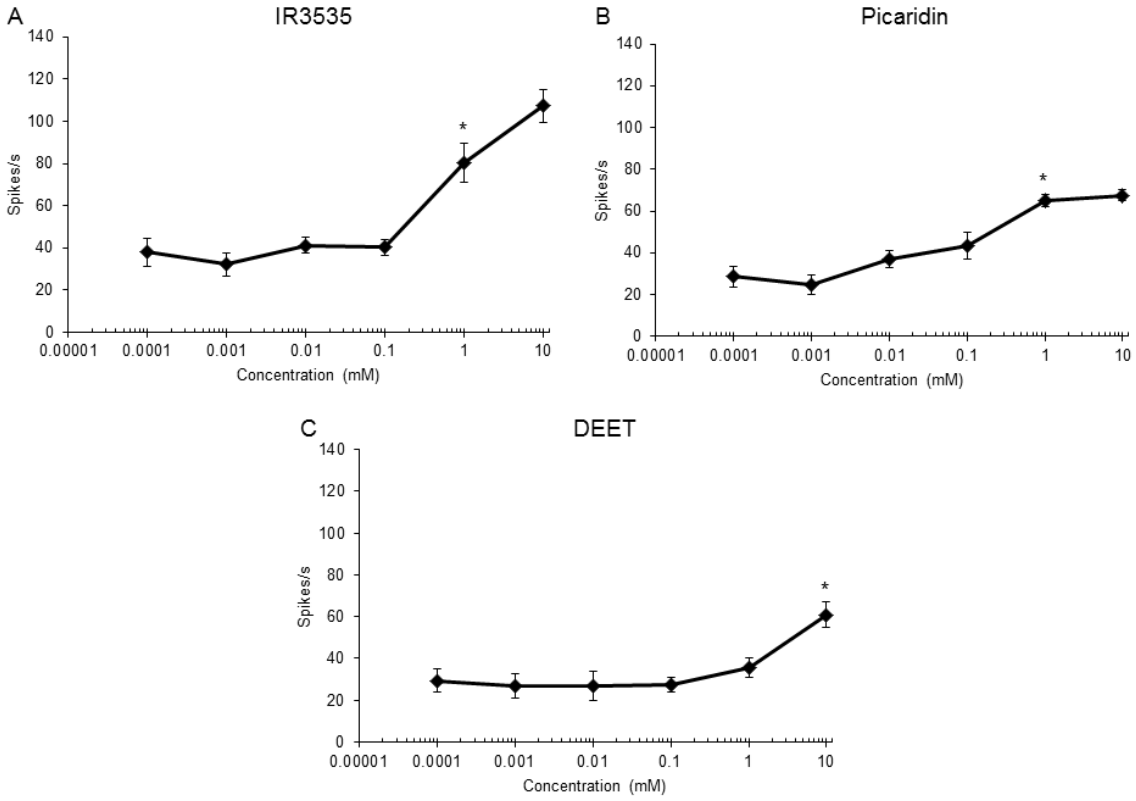
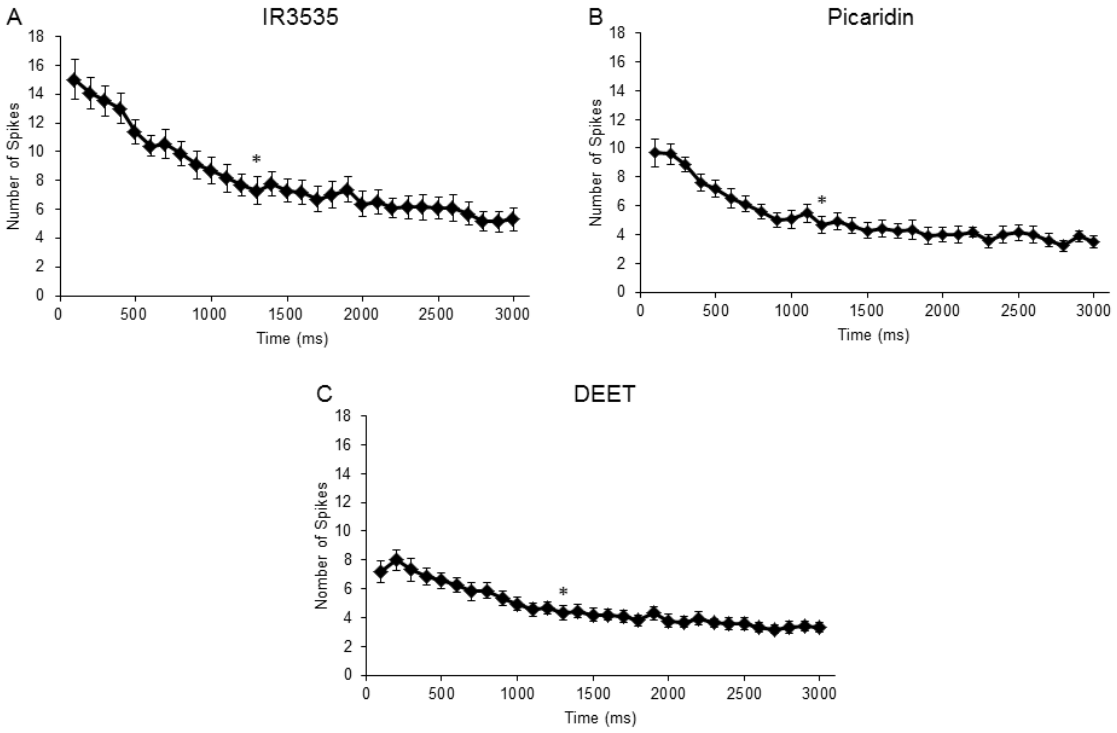

A
IR3535
Spikes/s
Concentration (mM)
B
Picaridin
Spikes/s
Concentration (mM)
C
DEET
Spikes/s
Concentration (mM)

Figure 5


A
IR3535
Number of Spikes
Time (ms)
B
Picaridin
Number of Spikes
Time (ms)
C
DEET
Nomber of Spikes
Time (ms)

LITERATURE CITED

BAR-ZEEV, M. AND SCHMIDT, C. H. 1959. Action of a repellent as indicated by a radioactive tracer. Journal of Economic Entomology 52: 268-269.

BERNAYS, E.A., R.F., CHAPMAN, AND SINGER M.S. 2000. Sensitivity to chemically diverse phagostimulatns in a single gustatory neuron of a polyphagous caterpillar. Journal of Comparative Physiology. 186: 13-19.

BOECKH, J., BREER, H., GEIER, M., ET AL. 1996. Acylated 1,3-aminopropanols as repellents against bloodsucking arthropods. Pesticide Science 48: 359-373.

BOHBOT, J. D. and DICKENS, J. C. 2010. Insect repellents: Modulators of mosquito odorant receptor activity. PLOS ONE 5: e12138. doi:10.1371/journal.pone.0012138.

BOHBOT, J. D. and DICKENS, J. C. 2011. Multiple activities of insect repellents on odorant receptors in mosquitoes. Medical and Veterinary Entomology 25: 436-444.

BOHBOT, J. D. and DICKENS, J. C. 2012. Odorant receptor modulation: Ternary paradigm for mode of action of insect repellents. Neuropharmacology 62: 2086-2095.

BROWN, M. AND HEBERT, A. A. 1997. Insect repellents: An overview. Journal of the American Academy of Dermatology 36: 243-249.

de BRYUNE, M., CLYNE, P. J., AND CARLSON, J. R. 1999. Odor coding in a model olfactory organ: The maxillary palp. The Journal of Neuroscience 19: 4520-4532.

CHAUHAN, K. R., KLUN, J. A., DEBBOUN, M., AND KRAMER, M. 2005. Feeding deterrent effects of catnip oil components compared with two synthetic amides against *Aedes aegypti.* Journal of Medical Entomology 42: 643-646.

COOK, J. I., MAJEED, S., IGNELL, R., PICKETT, J. A., BIRKETT, M. A., AND LOGAN, J. G. 2011. Enantiomeric selectivity in behavioural and electrophysiological responses of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. Bulletin of Entomological Research 101 (5): 541-550.

DAVIS, E.E. 1985. Insect repellents: Concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 22: 237-243.

"Dengue and the *Aedes aegypti* mosquito." National Center for Emerging and Zoonotic Infectious Diseases. Web. 18 Sep 2013. <http://www.cdc.gov/dengue/resources/30Jan2012/aegyptifactsheet.pdf>.

DESCOINS JR., C. AND MARION-POLL, F. 1999. Electrophysiological responses of gustatory sensilla of *Mamestra brassicae* (Lepidoptera, Noctuidae) larvae to three ecdysteriods: ecdysone, 20-hydroxyecdysone and ponasterone A. Journal of Insect Physiology. 45: 871-876.

DETHIER, V. G. 1960. The designation of chemicals in terms of the responses they elicit from insects. Journal of Economic Entomology 53: 134-136.

DICKENS, J. C., HART, W. G., LIGHT, D. M., et al. 1988. Tephritid olfaction: Morphology of the antennae of four tropical species of economic importance (Dipteral: Tephritidae). Annals of the Entomological Society of America 81: 325-331.

DICKENS, J.C. AND BOHBOT, J. D. 2013. Mini review: Mode of action of mosquito repellents. Pesticide Biochemistry and Physiology. 106: 149-155.

DITZEN, M., PELLEGRINO, M., AND VOSSHALL, L.B. 2008. Insect odorant receptors are molecular targets of the insect repellent DEET. Science 319: 1838-1841.

DYER, L. A., DODSON, C. D., STIREMAN III, J. O., et al. 2003. Synergistic effects of three *Piper* amides on generalist and specialist herbivores. Journal of Chemical Ecology. 29: 2499 – 2514.

GALINDO, K. AND SMITH, D. P. 2001. A large family of divergent drosophila odorant-binding proteins expressed in gustatory and olfactory sensilla. Genetics. 159: 1059-1072.

GHANINIA, M., IGNELL, R., AND HANSSON, B. S. 2007. Functional classification and central nervous projections of olfactory receptor neurons housed in antennal trichoid sensilla of female yellow fever mosquitoes, *Aedes aegypti*. European Journal of Neuroscience 26: 1611-1623.

GHANINIA, M., LARSSON, M., HANSSON B.S., AND IGNELL, R. 2008. Natural odor ligands for olfactory receptor neurons of the female mosquito *Aedes aegypti*: use of gas-chromatography-linked single sensillum recordings. Journal of Experimental Biology 211: 3020-3027.

GLENDINNING, J. I., DAVIS, A., AND RAI, M. 2006. Temporal coding mediates discrimination of "bitter" taste stimuli by an insect. The Journal of Neuroscience. 26: 8900-8908.

GLENDINNING, J. I. AND HILLS, T. T. 1997. Electrophysiological evidence for two transduction pathways within a bitter-sensitive taste receptor. Journal of Neurophysiology. 78:734-745.

GOTHILF, S., GALUN, R., AND BAR-ZEEV, M. 1971. Taste reception in the Mediterranean fruit fly: electrophysiological and behavioural studies. Journal of Insect Physiology 17: 1371-1384.

HILL, S. R., HANSSON B.S., AND IGNELL, R. 2009. Characterization of antennal trichoid sensilla from female southern house mosquito, *Culex quinquefasciatus* Say. Chemical Senses 34: 231-252.

HIROI, M., MARION-POLL, F., TANIMURA., T. 2002. Differentiated response to sugars among labellar chemosensilla in *Drosophila*. Zoological Science. 19: 1009-1018.

HODGSON, E. S., LETTVIN, J.Y., AND K.D. ROEDER. 1955. Physiology of a primary chemoreceptor unit. Science 122: 417-418.

HODGSON, E. S. AND ROEDER, K. D. 1956. Electrophysiological studies of arthropod chemoreception. I. General properties of the labellar chemoreceptors of Diptera. Journal of Cellular and Comparative Physiology. 28: 51-75. doi: 10.1002/jcp.1030480104.

IGNELL, R., OKAWA, S., ENGLUND, J. E., et al. 2010. Assessment of diet choice by the yellow fever mosquito *Aedes aegypti*. Physiological Entomology 35: 274-286.

ISONO, K. AND MORITA, H. 2010. Molecular and cellular designs of insect taste receptor system. Frontiers in Cellular Neuroscience 4: 1-16. doi: 10.3389/fncel.2010.00020

KATZ, T. M., MILLER, J. H., AND HEBERT, A. A. 2008. Insect repellents: Historical perspectives and new developments. Journal of the American Academy of Dermatology. 58: 865-871.

KIJIMA, H., NAGATA, K., NISHIYAMA, A., AND MORITA, H. 1998. Receptor current fluctuation analysis in the labellar sugar receptor of the fleshfly. Journal of General Physiology 91: 29-47.

KLUN, J.A., KHRIMIAN, A., AND DEBBOUN, M. 2006. Repellent and deterrent effects of SS220, picaridin, and deet suppress human blood feeding by *Aedes eagypti, Anopheles stephensi, and Phlebotomus papatasi.* Journal of Medical Entomology 43: 34-39.

KWON, Y., KIM, S.H., RONDEROS, D. S., ET AL. 2010. Drosophila TRPA1 channel is required to avoid the naturally occurring insect repellent citronellal. Current Biology 20: 1672-1678.

LANCE, D. AND BARBOSA, P. 1982. Host tree influences on the dispersal of late instar gypsy moth, *Lymantria dispar*. Oikos Journal of Ecology. 38: 1-7.

LEE, R. M. K. W., and CRAIG, D. A. 2009. Fine structure of the sense organs of the labella and labium of the mosquito *Aedes aeypti* (L.). The Open Entomology Journal 3: 7-17.

LEE, Y., KIM, S.H., AND MONTELL C. 2010. Avoiding DEET through insect gustatory receptors. Neuron 67: 555-561.

LEONARD, D. E. 1974. Recent developments in ecology and control of the gypsy moth. Annual Review of Entomology. 19:197-229.

LEONARD, D. E. 1984. Bioecology of the gypsy moth. In: Doane CC, McManus ML (eds) The gypsy moth: research toward integrated pest management. United States Department of Agriculture, Washington, DC, pp 9-29.

LICCIARDI, S., HERVE, J. P., DARRIET, F., ET AL. 2006. Lethal and behavioural effects of three synthetic repellents (DEET, IR3535, and KBR 3023) on *Aedes aegypti* mosquitoes in laboratory assays. Medical and Veterinary Entomology 20: 288-293.

LISCIA, A., MURONI, P., PIETRA, P. et al. 1995. Variations in salt sensitivity related to type and position of labellar chemosensilla in *Protophormia terrenovae*. Comparative Biochemistry and Physiology 112A: 335-344.

LISCIA, A., MAJONE, R., SOLARI, P., et al. 1998. Sugar response differences related to sensillum type and location on the labella of *Protophormia terraenovae*: a contribution to spatial representation of the stimulus. Journal of Insect Physiology 44: 471-478.

LISCIA, A. AND SOLARI, P. 2000. Bitter taste recognition in the blowfly: electrophysiological and behavioral evidence. Physiology and Behavior 70: 61-65.

MAES, F. W. AND VEDDER, C. G. 1978. A morphological and electrophysiological inventory of labellar taste hairs of the blowfly, *Calliphora vicina*. Journal of Insect Physiology 24: 667-672.

MARTIN, T. AND SHIELDS, V. 2012a. An electrophysiological analysis of the effect of phagostimulant mixtures on the responses of a deterrent-sensitive cell of gypsy moth larvae, *Lymantria dispar* (L.). Arthropod-Plant Interactions 6(2): 259-267.

MARTIN, T. L. AND SHIELDS, V. D. C. 2012b. Detection of alkaloids and carbohydrates by taste receptor cells of the galea of gypsy moth larvae, *Lymantria dipsar* (L.). Arthropod-Plant Interactions 6(4): 519-529.

MCIVER, S. AND SIEMICKI, R. 1984. Fine structure of pegs on the maxillary palps of adult *Toxorhynchites brevipalpis* theobald (Dipteral: Culicidae). International Journal of Insect Morphology and Embryology 13: 11-20.

MURAKAMI, M. and KIJIMA, H. 2000. Transduction ion channels directly gated by sugars on the insect taste cell. Journal of General Physiology 115: 455-466.

NATARAJAN R., BASAK, S. C., BALABAN, A. T., et al. 2005. Chirality index, molecular overlay and biological activity of diastereoisomeric mosquito repellents. Pest Management Science. 61: 1193-1201.

PALUCH, G., BARTHOLOMAY, L., AND COATS, J. 2010. Mosquito repellents: a review of chemical structure diversity and olfaction. Pest Management Science 66:925-935.

PAPPAS, L. G. and LARSEN, J. R. 1976. Gustatory hairs on the mosquito, *Culiseta inornata*. Comparative Physiology and Biochemistry 196: 351-360. doi: 10.1002/jez.1401960309.

PARK, B., LEE, S., CHOI, W., et al. 2002. Insecticidal and acaricidal activity of pipernonaline and piperoctadecalidine derived from dried fruist of *Piper longum* L. Crop Protection. 21: 249-251.

PITTS, R. J. AND ZWIEBEL, L. J. 2006. Antennal sensilla of two female anopheline sibling species with differing host ranges. Malaria Journal 5: 26. Doi:10.1186/1475-2875-5-26.

RHARRABE, K., SAYAH, F., AND MARION-POLL, F. 2011. Gustatory perception of phytoecdysteriods in *Plodia interpunctella* larvae. Entomologia Experimentalis et Applicata. 138: 33-39.

RICHARDS, L .A., DYER, L. A., SMILANICH, A. M., et al. 2010. Synergistic effects of amides from two *Piper* species on generalist and specialist herbivores. Journal of Chemical Ecology. 36: 1105-1113.

ROBERTSON, H. M., WARR, C. G., AND CARLSON, J. R. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of the United States of America 100: 14537-14542.

SANFORD, J. L., SHIELDS, V. D. C., AND DICKENS, J. C. 2013. Gustatory receptor neuron responds to DEET and other insect repellents in the yellow-fever mosquito, *Aedes aegypti*. Naturwissenschaften. 100: 269-273. doi 10.1007/s00114-013-1021-x.

SATO, K., TANAKA, K., AND TOUHARA, K. 2011. Sugar-regulated cation channel formed by an insect gustatory receptor. Proceedings of the National Academy of Sciences of the United States of America 108: 11680-11685.

SCHOONHOVEN, L. M. AND DETHIER, V. G. 1966. Sensory aspects of host-plant discrimination by lepidopterous larvae. Archives Néerlandaises de Zoologie. 16: 497-530.

SCHOONHOVEN, L. M. AND LIN-ER, L. 1994. Multiple mode of action of the feeding deterrent, toosendanin, on the sense of taste in *Pieris brassicae* larvae. 175: 519-524.

SCOTT, K., BRADY., R., CRAVCHIK., A. 2001. A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. Cell 104: 661-673.

Shields, Vonnie D.C. and Martin, Timothy L. 2011. The Structure and Function of Taste Organs in Caterpillars. *In:* Lynch, E.J. and Petrov, A.P. (eds.), The Sense of Taste, Nova Science Publishers, Inc. Hauppauge, NY. Chapter 11, pp. 147-166.

SHIELDS, V.D.C. and MITCHELL, B.K. 1995. Responses of maxillary styloconic receptors to stimulation by sinigrin, sucrose, and inositol in two crucifer-feeding polyphagous lepidopterous species. Philosophical Transactions of the Royal Society of London. B. 347: 447-57.

SHIELDS, V.D.C. 2003. Ultrastructure of Insect Sensilla. *Encyclopedia of Entomology*, Kluwer Academic Publishers, pp. 2407-2420.

SHIELDS, V. D. C., RODGERS, E. J., ARNOLD, N. S., et al. 2006. Feeding responses to selected alkaloids by gypsy moth larvae, *Lymantria dispar* (L.). Naturwissenschaften. 93: 127-130.

SHIELDS, V. D. C., SMITH, K. P., ARNOLD, N. S., et al. 2008. The effect of varying alkaloid concentrations on the feeding behavior of gypsy moth larvae, *Lymantria dispar* (L.) (LepidopteraL Lymantriidae). Arthropod Plant Interactions. 2: 101-107.

SHIELDS, V. D. C. 2009. Fine structure of the galeal styloconic sensilla of larval *Lymantria dispar* (Lepidoptera: Lymantriidae). Annals of the Entomological Society of America. 102: 1116-1125.

STOFFOLANO, J. G., ANGIOY, A. M., CRNJAR, R. et al. 1990. Electrophysiology recordings from the taste chemosensilla of *Tabanus nigrovittatus* (Diptera: Tabanidae) and determination of behavioral tarsal acceptance thresholds. Journal of Medical Entomology 27: 14-23.

SYED, Z. AND LEAL, W. S. 2007. Maxillary palps are broad spectrum odorant detectors in *Culex quinquefasciatus*. Chemical Senses 32: 727-738. doi: 10.1093/chemse/bjm040

SYED, Z. AND LEAL, W. S. 2008. Mosquitoes smell and avoid the insect repellent DEET. Proceedings of the National Academy of Sciences of the United States of America 105: 13598-13603.

SYED, Z., PELLETIER, J., FLOUNDERS, E., ET AL. 2011. Generic insect repellent detection from the fruit fly *Drosophila melanogaster*. PLOS ONE. 6: e17705. doi:10.1371/journal.pone.0017705

TAVARES, W. S., CRUZ, I., PETACCI, F., et al. 2011. Insecticide activity of piperine: Toxicity to eggs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Diatraea saccharalis* (Lepidoptera: Pyralidae) and phytotoxicity on several vegetables. Journal of Medicinal Plant Research. 5: 5301-5306.

VAN LOON, J. J. AND SCHOONHOVEN, L. M. 1999. Specialist deterrent chemoreceptors enable *Pieris* caterpillars to discriminate between chemically different deterrents. Entomologia Experimentalis et Applicata. 91: 29-35.

VOSSHALL, L.B. AND STOCKER, R. F. 2007. Molecular architecture of smell and taste in *Drosophila.* Annual Review of Neuroscience. 30: 505-533.

XIA, Y., WANG, G., BUSCARIOLLO, D. et al. 2008. The molecular and cellular basis of olfactory-driven behavior in *Anopheles gambiae* larvae. Proceedings of the National Academy of Sciences. 105: 6433-6438.

ZHOU, D., VAN LOON, J. J., AND WANG, C. Z. 2010. Experience-based behavioral and chemosensory changes in the generalist insect herbivore *Helicoverpa armigera* exposed to two deterrent plant chemicals. Journal of Comparative Physiology A. 11: 791-799.

CURRICULUM VITAE

NAME: Jillian Sanford
Current Address: Department of Biological Sciences, 8000 York Rd., Towson University, Towson, MD 21252
Phone: (301) 785-0228
Email: jsanfo2@students.towson.edu

GRADUATE EDUCATION

Towson University
Degree: Master of Science
Major: Biology
Current GPA: 3.83
Anticipated Graduation Date: 12/2013

UNDERGRADUATE EDUCATION

Towson University
Degree: Bachelor of Science
Majors: Molecular Biology, Biochemistry, and Bioinformatics; Biology
Minor: Chemistry
Cum laude
University Honors
GPA: 3.68

WORK EXPERIENCE

- Graduate Teaching Assistant – Department of Biological Sciences, 8000 York Rd, Towson University, Towson MD, 21252. (8/2012 – Present)
    - Teach two, three hour lab courses a week consisting of 24 students each
    - Conduct open study sessions once a week to assist students with coursework
    - Assist professor with tasks related to the course including construction of lab exams and grading of exams to monitor student performance
    - Perform routine maintenance of a colony of *Lymantria dispar*, including preparation of insect diet and separation of insects into different instars

- Biological Science Technician – Henry A. Wallace Beltsville Agricultural Research Center, ARS, USDA. (6/2011 - 8/2012)
    - Conduct neurophysiological recordings on the target organism of a research project, namely the mosquito species, *Aedes aegypti*
    - Calculate and perform dilutions from stock materials to make solutions used for testing of research subjects
    - Occasional assistance of maintenance of a colony of *A. aegypti*, including sorting of pupal stages by developmental period

- Student Research Assistant – Department of Biological Sciences, 8000 York Rd, Towson University, Towson MD, 21252. (6/2009 – Present)

    - Perform maintenance of a colony of *Lymantria dispar*, the target insect research organism for the laboratory
    - Perform behavioral and neurophysiological assays on *L. dispar* as part of the overall research goals of the laboratory
    - Perform data analysis using Microsoft excel and the statistical analysis program, NCSS

RESEARCH PROJECTS

- 8/2012 – Present – Electrophysiological examination of the response of the medial styloconic sensillum in the gypsy moth larvae, *L. dispar*, to insect repellents
- 7/2011-8/2012 – Electrophysiological examination of the responses of a labellar sensillum in the mosquito species, *A. aegypti*, to insect repellents
- 8/2010-present – Behavioral response of Colorado potato beetle to lights of varying wavelengths
- 8/2010-present – Behavioral response of gypsy moth larvae (*L.dispar*) to lights of varying wavelengths
- 6/2009-5/2011 – Habituation of gypsy moth larvae (*L. dispar*) to various alkaloid compounds

PUBLICATIONS

Sanford JL, Shields VDC, and Dickens JC (2013) Gustatory receptor neuron responds to DEET and other insect repellents in the yellow-fever mosquito, *Aedes aegypti*. *Naturwissenschaften*. 100: 269-263. DOI: 10.1007/s00114-013-1021-x

TECHNICAL SKILLS

Software - Microsoft office, NCSS 2007, GraphPad PRISM5, R

COURSEWORK	SEMESTERS
(U = Undergraduate; G = Graduate)	
Analytical Chemistry + Lab (U)	1
Applied Biotechnology (G)	1
Biochemistry (U)	1
Biodiversity (U)	1
Bioethics (U)	1
Bioinformatics + Lab (U)	1
Calculus for Applications (U)	1
Cell Biology (U)	1
General Physics; Non Calculus Based (U)	2
Gene Expression and Regulation (G)	1

General Chemistry + Lab (U)	2
Genetics (U)	1
Human Anatomy and Physiology + Lab (U)	2
Immunology (U)	1
Mechanisms in Animal Physiology (G)	1
Molecular Biology + Lab (U)	1
Molecular Biology (G)	1
Organic Chemistry + Lab (U)	2
Professional Aspects of Biology (G)	1
Virology (G)	1

ORAL/POSTER PRESENTATIONS

- Towson University 13th Annual Student Research Scholarship Expo, Towson, Maryland (4/2013)
- 292nd Maryland Entomological Society Regular Meeting, University of Maryland Baltimore County, Baltimore, MD (4/19/2013)
- 10th International Congress of Neuroethology, University of Maryland, College Park, MD USA (8/5/2012-8/10/2012)
- Towson University 12th Annual Student Research and Scholarship Expo, Towson, Maryland (4/2011)
- Experimental Biology 2011, Washington D.C. (4/2011)
- Towson University 11th Annual Student Research and Scholarship Expo, Towson, Maryland (4/2010)

AWARDS, GRANTS, and HONORS

- Towson University Graduate Student Association Award – $500 (10/2012)
- Towson University Honors Scholarship – $1000 annually (8/2007-5/2011)
- Towson University Undergraduate Research Grant – $ 500 (2009, 2011)
- Towson University FCSM Undergraduate Research Grant – $ 500 (2009, 2011)
- Towson University Dean's List – (1/2008-5/2008, 1/2009-5/2011)
- John David Horst Memorial Award for Outstanding Undergraduate Research Project –$1270 (2011)