Comparison of Male and Female Electroantennogram Responses to a Panel of Plant Associated Volatiles in the Common House Cricket, *Acheta domesticus*

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THESIS APPROVAL PAGE

This is to certify that the thesis prepared by **Mary Catherine Neslund** entitled **"Comparison of Male and Female Electroantennogram Responses to a Panel of Plant Associated Volatiles in the Common House Cricket, *Acheta domesticus*"**, has been approved by her committee as satisfactory completion of the requirement for the degree of Master of Science.

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Abstract

Electroantennogram (EAG) responses were recorded from both sexes of the common house cricket, *Acheta domesticus* to a panel of ecologically relevant volatiles. A panel of 11 volatiles in males p cresol, anisole, sabinene, methyl salicylate, heptanal, 2 acetyl furan, tri ethyl amine, m cresol, cis-3-hexen-1-ol, n undecane, 2 methyl butyl benzoate , and 10in females 2 acetyl furan, 2 methyl butyl benzoate, tri ethyl amine, methyl salicylate, sabinene, p cresol, iso eugenol, methyl eugenol, eugenol, and m cresol were tested. They represented the chemical classes: monoterpenoid, aromatic ester, aromatic alcohol, aromatic ether, fatty acid derivative, green leaf volatile, and furan. Overall, I found that compounds from the aromatics group elicited the most robust EAG responses. The aromatic ester, 2-methyl butyl benzoate was found to elicit the largest EAG responses in males, while the furan compound, 2-acetyl furan, elicited the largest EAG responses in females. Dose-response experiments were carried out with two of the compounds (i.e., *m*-cresol and 2-acetyl furan) that elicited robust EAG responses in both sexes. The concentrations tested spanned a total of more than 2 log units for each compound. I found that the antennae of both sexes responded to 2-acetyl furan and *m*-cresol in a dose-dependent manner. The threshold concentration for both sexes and both compounds was 50 µg. For 2-acetyl furan, the normalized EAG value observed in males at this dose was 0.30 mV and 0.34 mV in females. For *m*-cresol, these values were 0.54 mV in males and 0.25 mV in females. For 2-acetyl furan and *m*-cresol, the maximum normalized EAG values observed at the highest dose tested (i.e., 5000 µg) were 0.73 mV for males and 1.02 mV for females and 1.02 mV for males and 0.70 mV for females, respectively.

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Introduction

Acheta domesticus

Acheta domesticus, the common house cricket, is believed to have migrated from northern Africa or southwestern Asia (Ghouri, 1961). These insects are considered to be hemimetabolous, bearing nymphal stages resembling that of the adult form. As adults, these winged insects measure 3/4" to 1" long, are yellowish-brown in color, and bear three dark bands on the head. They are also endowed with a pair of antennae, which act as their "nose" to detect odorants in the environment. During warm weather, they often populate under rocks, lumber, piles of debris, and garbage dumps. With the onset of colder weather, they move into warmer locations, such as peoples' homes, where they seek shelter by concealing themselves in darkly lit areas, such as in basements, cracks in walls, and behind baseboards (Ghouri, 1961). In nature, they are considered to be omnivorous, since they feed on a very wide variety of foods, including plants, such as wheat, grain, oil seed, etc., as well as other insects and decaying matter (Ghouri, 1957). House crickets are nocturnal and remain hidden during the day. Inside the home, house crickets are generally a nuisance, as they feed and damage clothing, carpets, draperies, and upholstery, in addition to paper products, rotting fruit, and other organic matter. In order to attract female conspecifics, male house crickets make loud chirping sounds which are often very annoying to many home owners and may result in disturbances in sleep patterns.

This study is the first to investigate the chemosensory response of the house cricket to a panel of plant-associated volatiles and as such, should begin to help contribute to our understanding of cricket neurophysiological activity and how this may relate to food-finding and selection by this insect.

Odor-guided behavior in insects

Olfactory stimuli play an important role in the orientation of many animals in their environment, including insects. Olfaction is the principal sensory modality by which insects locate their food sources, mates, and oviposition sites. For foraging purposes, crickets have to be able to detect plant-associated volatiles (odorants). In order to gain insights into the mechanisms underlying odor-mediated orientation and the interaction of insects with those plants, it is necessary to study how plant volatiles are detected, discriminated, and processed by the olfactory system of the insect. Crickets, like other insects, bear paired multi-segmented antennae, their main olfactory organs, which allow them to detect these odorants; many insects also bear additional olfactory organs on their labial, maxillary palps, and/or feet.

The neural organization of the olfactory systems of insects is surprisingly similar to those of mammals and other vertebrates (Hildebrand and Shepherd 1997). Both invertebrates and vertebrates are capable of detecting and discriminating among a large number of odorants that differ in size, shape, and complexity (Hildebrand and Shepherd 1997). Based on this similarity, many investigators have decided to study the insect olfactory system as a valuable model to reveal general mechanisms of olfaction, odor-induced behavior, synaptic processing and coding of odorant information, neural plasticity, learning and memory (Strausfield and Hildebrand. 1999; Menzel 2001; Heinbockel et al., 2013). A major advantage of insects as models for olfactory research and neuroscience in general is their relatively simple peripheral and central nervous system compared with their vertebrate counterparts (Haupt et al. 2010). Their moderately complex nervous system gives rise to a rich behavioral repertoire, while the cost of

maintaining them as experimental animals is low. Our understanding of insect brains has benefitted from individually identifiable neurons and small networks as functional units (Haupt et al. 2010).

Olfactory Organs (in insects), sensillum, types and function

A morphological study of the antennal sensilla by Shields et al., (2014; in preparation) revealed that house cricket antennae bear at least four putatitve olfactory sensillum types: three basisonic (pegs) and one coeloconic (peg within a deep pit). The cuticular surface of these sensilla is pierced by numerous small pores (called multiporous sensilla), which allows the insect to detect diverse odorants and to perceive its external environment (Altner et al., 1977; Steinbrecht, 1980; Shields and Hildebrand, 2001). Odor molecules must first reach the surface of the olfactory sensillum and then find their way through the cuticular pores to the underlying sensory neurons, where they bind to specific receptor sites on the dendrites of olfactory receptor cells (ORCs). Multiporous sensilla are typically innervated by 1-5 ORCs which are bipolar neurons that project an axon, each, to the antennal lobe (AL), as well as branched dendrites into the sensillar lymph of the sensillum (Keil, 1999). Here, the ORCs transduce the chemical stimuli into electrophysiological responses. Information concerning the olfactory stimulus, encoded as patterns of action potentials resulting from the transduction process, is conveyed along axons of the ORCs to the brain.

Each ORC usually expresses only one odorant receptor (OR), a seven transmembrane protein receptor domain that is located in the dendrites and comes into direct contact with the odorant (Buck and Axel, 1991). In insects, it is hypothesized that

this domain is inverted within the plasma membrane, with the C-terminal located outside and the N-terminal located inside. This domain of ligand and receptor forms a heteromeric complex with a chaperone protein, Orco (formerly known as OR83b in *Drosophila*). Orco is thought to help in transporting the OR to the dendritic interface from the ORC soma and also helps in forming the receptor complex for the odorant (Benton et al, 2006). ORCs expressing similar ORs bear axons which project to the AL and synapse in the same glomerulus (Clyne et al, 1999). In the glomeruli, synaptic connections are formed with projection neurons (PNs) which send action potentials to higher order processing centers in the brain, such as the lateral horn of the protocerebrum and, subsequently, can trigger odor guided behavior (Boeckh and Tolbert 1993).

Odorant Transduction

As odorants enter the sensillum through small pores in the cuticle, it is thought that pore tubules direct the transport of these typically hydrophobic odorants (Keil, 1999). Odorant binding proteins (OBPs) are assumed to be secreted by tormogen and trichogen sheath cells surrounding the ORCs and are thought to come into contact with, and bind to, the odorants, aiding in their transport from the sensillum lymph to the ORC dendrites (Vogt and Riddiford, 1981). Although OBP genes have also been identified in vertebrates, the gene family found in insects is highly conserved and exclusive (Clyne et al, 1999). While OR genes are present in vertebrates and nematodes, they show no homology to the ORs identified in insects which represent a large highly divergent set of genes (Vosshall et al, 1999). Although there is some debate over the number of OR

genes ranging from 10-400 across insect orders, it is generally accepted that OR gene loss and gain is an evolutionary adaptation to available resources (McBride et al., 2007).

In the well-studied mammalian model of olfaction, odorants binding to ORs activate a G protein coupled receptors (GPCRs). Activation of the G protein receptor, G_{olf}, allows intracellular levels of the second messengers IP_3 and cAMP to rise and to activate cyclic nucleotide-gated (CNG) channels to open (Hildebrand and Shepherd, 1997). This intracellular increase in cations will then produce an action potential at the axon hillock and the signal will be propagated to higher brain centers. Insect olfactory pathways, however, are less understood, as there is still some debate with respect to the transduction cascade mechanism. In *Drosophila* components of the cAMP pathway and IP_3 pathway are present in the olfactory system (Vosshall et al, 2000). In vivo recordings from antennal neurons in *Drosophila* showed action potentials being propogated with the application of IP_3 (Stengl, 1993; Tallura et al., 1995). Knockout experiments of the *Drosophila* G protein receptor (G_q) gene showed alteration in the ORCs odor-mediated responses. Peculiarly these experiments did not show any complete anosmic responses as would have been expected if, in fact, insect olfactory pathways were GPCRs (Kain et al, 2008).

Recent electrophysiological experiments have provided evidence that insect ORs are a ligand-gated non-specific cation channels (Sato et al, 2008). In Sato's model the specific OR forms the (aforementioned) heteromeric complex with the chaperone Orco. When an odorant is bound to this complex, it triggers the formation of a channel that is cation permeable to cause a depolarization. This theory was challenged though by Wicher et al (2008) who proposed an ion channel –GPCR hypothesis. In Wicher's model

the OR still forms a heteromeric complex with Orco, but binding of the odorant to the OR causes a G protein cascade which activates adenyl cyclase (AC) to increase intracellular cAMP. This increase then causes a CNG-like channel (Orco) to open. Although both studies offer feasible explanations for the activation pathway of insect olfaction, more studies must be performed to determine which pathway is correct or if it is a blend of the two proposed here. It is important though to note that this new information provides researchers a strategy to investigate novel insect repellents, which will have little to no effect on humans (Pellegrino and Nakagawa, 2009).

Olfactory Coding

Insects have the ability to discriminate thousands of odorants in the environment (Hildebrand and Shepherd, 1997). Processing and discrimination of odors mostly takes place in the central nervous system (CNS), although peripheral ORCs play an important role in the detection, identification, and discrimination of odor molecules. To understand odor coding it is therefore essential to classify ORCs which are activated by specific odors (Malnic et al, 1999). Specialized ORCs, responding to host volatiles, have been identified in many insect species including moths (Todd and Baker, 1993), silkworms (Tanaka et al, 2009), and mosquitoes (Carey et al., 2010), while other ORCs seem to be more broadly tuned to odors found in the environment (Carey et al., 2010; Hallem and Carlson, 2006). Labeled-line odor detection, in which one odor molecule activates one glomerulus, is associated with detection of host volatiles and is the most selective. This odor coding scheme is associated with more innate behaviors such as pheromone detection in male moths (Hansson and Christensen, 1999). Across fiber patterning, when

ORCs respond more broadly to a range of compounds, appear to be associated with plant and oviposition related compounds (Davis, 1976; Dethier, 1976). Neither of these odor detection schemes can completely describe odor coding in insects so a novel idea was presented by Malnic et al (1999). In this new hypothesis distinct ORCs are broadly tuned to a set of compounds with differential affinities. Affinity is determined by the defining characteristics of the odorant molecules such as shape, size, and hydrophobicity of functional groups (Ignell and Hansson, 2005). ORCs can then generate a differential response profile to various odorants, additionally many ORCs can be activated with differential affinities for the compound (Hallem and Carlson, 2006).

Antennal Lobe Evolution

The antennal lobe is homologous to the olfactory bulb in vertebrates; ORCs which express the same OR, converge onto the same glomerulus. These glomeruli, normally 50-200, are housed in the antennal lobe and will send projection neurons to higher brain centers for odorant processing (Vosshall et al, 2000). When an odorant comes into contact with the antennae, many ORCs may be activated and send signals to several different glomeruli in the AL. A glomerulus serves as an address for specific features of an odorant stimulus and different features are encoded in different glomeruli. Buck and Axel (1991) first discovered this "combinatorial code" concept in mammals that allows fairly small numbers of ORs (60 in *Drosophila*) to be used by the CNS to classify and interpret thousands of different odorants.

Orthopteran antennal lobe evolution reveals trends about how AL complexity might have evolved over time. Six species of orthopterans were studied from a variety of

groups that spanned complex to simple AL structure. It was found that the basal suborder Ensifera (*Tettigonia viridissima*; green bush cricket) had glomerular structure which was similar to many insects with ~50 uniglomerular structures, whereas members of the more derived suborder Gryllidae, (*Gryllus bimaculaus*; southern field cricket, closely related to *A. domesticus*) had the beginnings of specialization with microglomeruli forming within the larger glomerular structure. More highly evolved suborders such as Caelifera, (*Schistocerca gregaria;* desert locust), showed the highest degree of specialization with thousands of microglomeruli making up the AL (Ignell et al, 2001). Although no concrete reasons are known for this specialization, it has been speculated that more complex glomerular structure will allow greater resolution of odorants and increased coding capacity (Hansson and Stensmyr, 2011).

Electroantennography

In order to tease out which specific odorants elicit depolarizing electrophysiological responses, the antennae of *A. domesticus* can serve as a "biodetector" using the electroantennogram (EAG) technique to screen for biologically active components. This technique allowed me to obtain an overall bigger olfactory picture to record the electrical activity across an antenna by summating electrical potentials of many antennal ORCs which respond almost simultaneously to odorants by measuring the change in electrical potential between distal and proximal sections of the antennal flagellum (Olsson and Hansson, 2013). (Schneider, 1957; Kaissling, 1971; Nagai, 1981; Olsson and Hansson, 2013). In addition, this technique allowed me to assess the receptive range of the antenna.

Specific Aims

In order to analyze olfactory function in the cricket, *Acheta domesticus*, it is essential to know which specific odor stimuli play a role in the behavior of this insect. The main goal of my research was to determine which odorants elicited physiological responses from ORCs found on the antennae. As a first step, we (Huynh, Neslund, and Shields, 2014,unpublished data) carried out behavioral bioassays to determine which odorants, chosen from the habitat of the cricket (Ghouri, 1961; Otte and Cade, 1976; McFarlane and Alli, 1985; Dillman et al, 2012), elicited positive anemotaxic (orient towards) behavioral attraction (i.e., cricket walking toward the odorant source). We hypothesized that a subset of odorants would elicit positive anemotaxic behavioral attraction. More specifically, we used a Y tube olfactometer bioassay to carry out these experiments, where one arm of the Y-tube served as the control (i.e., no odorant present) and the other arm, as the test (i.e., test odorant was present) (after Blackmer et al, 2004; Horton and Landolt, 2007). Results were recorded only if the cricket walked down the main stem of the Y and into either arm of the Y-tube. Preliminary experiments from this behavioral bioassays using 103 odorants yielded a smaller panel that elicited positive responses. From this smaller panel of odorants tested (i.e., 10 odorants tested in females and 11 in males), we observed significant positive anemotaxic results. More specifically, when we compared the results for both males and females, we found seven odorants to be significantly attractive in both sexes (e.g., 2-acetyl furan, 2-methyl butyl benzoate, *m*-cresol, sabinene, and tri-ethyl-amine, *p*-cresol, and methylsalicylate), while four odorants elicited significant attractive responses only in males (e.g., anisole, *cis*-3-hexen-1-ol, *n*-

undecane, and heptanal) and three only in females (e.g., eugenol, isoeugenol, and methyleugenol).

Based on the aforementioned results, I performed EAG recordings to measure the receptivity of the cricket antennae by determining which specific odorants elicited depolarizing electrophysiological responses (Figs. 1, 2). I hypothesized that one or more of the four cricket olfactory sensillum types (i.e., basiconic types A, B, and/or C, coeloconic) would respond to one or several odorants, as a result of data obtained from the behavioral bioassays. In addition, I used the EAG technique to carry out dose-response experiments to determine the sensitivity of the antennae (Figs. 7,8). I hypothesized that *A. domesticus* would respond with high sensitivity to a few compounds and low sensitivity to several others. To date, much of the EAG research has been focused on pheromone identification (Baker et al., 1991). More recently, the focus has shifted to the detection of plant volatiles that are of behavioral relevance (i.e. attractants, repellents) (Raguso et al., 1996; Raguso and Light, 1998). The results of this research will hopefully advance our understanding of insect olfaction. Furthermore, it will allow us to determine which odorants, as a result of our behavioral bioassays, elicited an electroantennographic response by the antennal ORCs.

Materials and Methods

Animals

Randomly selected pre-adult crickets (Petco, Timonium, MD) (ca. 2.5 cm in length) were maintained on an artificial diet and gel water source (Fluker Port Allen, LA) at ca. 60% relative humidity under a 12L: 12D photoperiod regimen and superimposed 25-26 ˚C temperature cycle. Each un-mated pre-adult was then inspected prior to their selection to ensure that the antennae were intact. The crickets were naive to the test compounds prior to testing.

Test Compounds

Male and female crickets were challenged in a preliminary behavioral bioassay with a panel of 103 odorants from the ecological habitat of the cricket using a 500 μg/μl load for each odorant. The crickets were naive to the test compounds prior to testing. The odorants came from the following chemical classes: terpenoids (e.g., sabinene), aromatics (e.g., 2-methylbutylbenzoate), aliphatics (e.g., *cis*-3-hexenylpropionate), fatty acid derivatives (e.g., undecane), green leaf volatiles (e.g., *trans*-3-hexen-1-ol), nitrogen-bearing (e.g., 1-nitropentane), and furan (e.g., 2-acetylfuran). Each of the compounds has a chemical and isomeric purity >99% (Sigma Aldrich). Each of the compounds was diluted in mineral oil (light white oil, Sigma, St. Louis, MO) or hexane. All solutions were kept in vials, sealed with Parafilm, and stored in the refrigerator until use. Based on behavioral experiments, 10 odorants were selected for females and 11 for males for further EAG study. For females, these include: 2 acetyl furan, 2 methyl butyl benzoate, tri ethyl amine, methyl salicylate, sabinene, p cresol, iso eugenol, methyl eugenol, eugenol, and m cresol.

For males, these include: p cresol, anisole, sabinene, methyl salicylate, heptanal, 2 acetyl furan, tri ethyl amine, m cresol, cis-3-hexen-1-ol, n undecane, 2 methyl butyl benzoate.

EAG Recording

Two small diameter glass capillary pipettes were pulled to a fine point using a micropipette puller (Sutter Instrument Co. Novato, CA). These pipettes were then filled with an insect Ringer solution (ph =7.0-7.1) (Pichon, 1972) and both contain a silver wire, which serves to make contact between the amplifier and electrodes. One of the two pipettes was then placed into the isolated head of *A. domesticus*. The head was secured by a minimal amount of melted dental wax. This pipette served as the indifferent or ground electrode. After excision of 3-5 distal antennal segments, 1-2 mm of the antennae was inserted into the second glass pipette with the fine tip broken off just enough to accommodate the antennal tip, this served as the recording electrode. All odorants were dissolved in either mineral oil or hexane. The latter two compounds acted as our controls, as well as diluents, since neither compound was found to elicit significant EAG during our preliminary experiments. Approximately 24 h prior to each experiment, each odorant was prepared by depositing a 500µg aliquot on a small circular (5 mm) piece of filter paper and inserting it into the distal end of a Pasteur pipette and sealing the end with Parafilm. The odorants were then kept at 4 ˚C, overnight. The following day, odorants were allowed to equilibrate for 2 hours prior to use. A constant stream of charcoal-filtered and humidified air flowed at 1,000 ml/min (CS-05 stimulus controller unit, Syntech, the Netherlands) into an "L"- shaped tube (10 cm) positioned ca. 5 mm from the antenna prior to odor stimulation. In keeping with our preliminary results, individual odorants were tested using 500 µg, as this concentration

typically elicited robust responses for the majority of the odorants. To test individual odorants, the tip of the Pasteur pipette was placed in a small opening on the side of the L-shaped tube. A stimulus controller unit delivered 300 ml/min of odorant-laden air for 2 sec contained in the Pasteur pipette toward the antenna for testing. A glass funnel (3.5 cm i.d.) attached to an air evacuation line was positioned near the preparation to remove odor-bearing air following stimulus delivery. This process was repeated for each odorant, allowing for a two minute recovery period between stimulations. Signals were amplified 10x (Syntech Probe, Syntech, the Netherlands). The signal was further processed to produce an EAG signal using a PC-based signal-processing program EAG Pro (Syntech, Hilversum, the Netherlands). Individual odorants were delivered to the antenna and responses were measured as change in potential between baseline and maximum deflection. Male and female responses were recorded and normalized to a positive control. The control [standard] stimulus of 500µg of *trans*-3-hexen-1-ol produced an EAG of 0.3mV in both male and female whole head preparations. The response to the standard stimulus in mV was recorded and used as the denominator in the equation for normalization where the numerator was the response in mV to the volatile being tested. The mean values were determined for all normalized responses to a given volatile and plotted as normalized EAG response (Figs. 1, 2).

Mean normalized responses were calculated and compared to the panel of odorants tested using a repeated measure ANOVA followed by a Tukey's multiple comparisons test. Dose response experiments were then carried out using compounds that had elicited robust EAG responses (i.e., 2-acetyl furan and *m*-cresol for both males and females) from our preliminary results. The doses chosen were 5000 µg, 1000 µg, 500 µg, 100 µg, 50 µg, and

10 μg filter paper loadings, in keeping with our preliminary results. Mean normalized responses were calculated and compared using a repeated measure ANOVA followed by a Tukey's multiple comparisons test. Male and female EAG responses were compared using a Students *t-test* with a Bonferroni correction for multiple comparisons.

Results and Discussion

Food-Associated Volatiles

Within the olfactory system, omnivorous insects such as the house cricket, *A. domesticus*, can perceive food related volatiles and use them as chemical cues to locate food and oviposition sites (Visser, 1988). *Acheta domesticus* showed typical EAG depolarizations to all compounds tested in both males and females. This study showed that *A. domesticus* males and females showed EAG depolarizations (normalized mV) to a panel of plant volatiles in keeping with those found in its natural habitat (Figs. 1, 2), including monoterpenoids (sabinene) , aromatic esters (2 methyl butyl benzoate, methyl salicylate) , aromatic alcohols (eugenol, isoeugenol, *m*-cresol, *p*-cresol), aromatic ethers (anisole, methyl eugenol), fatty acid derivatives (*n*-undecane, heptanal), green leaf volatiles (cis-3-hexen-1-ol), and furan (2-acetyl furan).

The largest EAGs in females were observed to 2-acetyl furan (1.15, normalized). This compound naturally occurs in fermented substances such as apple cider and wine (Hu et al, 2013). Interestingly, yeast, which is present in both of the aforementioned substances, has been shown to increase fecundity and growth in *Acheta domesticus* (Ghouri, 1958). Yeast has also been shown to increase growth rates in other insect species, which indicates the presence of an unknown insect growth factor present (Lipke and Fraenkel, 1956). The largest EAGs in males were observed to 2-methylbutylbenzoate (1.18, normalizes), which is naturally occurring in *Hedychium coronarium* (white ginger lily) (Matsumoto et al, 2011). The white ginger lily has a wide distribution and is commonly considered to be a weed.

The response profile reveals high sensitivities of the antennal receptor system to the general aromatics. In the group of aromatic alcohols, *m*-cresol elicited similar responses in males and females (0.88 and 0.85, normalized respectively), while *p*-cresol elicited significantly different responses (0.87 and 0.52, normalized) in females and males, respectively; Students *t-test*, p=0.005, n=10). *M*-cresol has been found to be a component of the carabid beetle, *Pterostichus fortis*, defensive system (Kanehisa and Murase, 1977), while *p*-cresol is a component of oviposition attractant in eastern treehole mosquitoes, *Aedes triseriatus* (Bentley et al., 1981). The aromatic alcohols, eugenol and isoeugenol, and the aromatic ether, methyleugenol, elicited moderately high responses in females (0.86, 0.79, and 0.75 normalized, respectively). To the human nose these latter compounds have a clove-like odor and are associated with plant essential oils. Methyl eugenol has been found to be a potent attractant of the oriental fruit fly, *Bactrocera dorsalis* and has been used in lures (Vargas et al., 2000). In addition, isoeugenol and eugenol were found to be attractive to the northern corn root worm, *Diabrotica barberi* (Ladd et al., 1983). Interestingly, one or more compounds (i.e., eugenol, isoeugenol and methyleugenol) displayed contact toxicity to pests of stored grains (i.e., *Sitophilus zeamais*, *Sitophilus granarius*, *Tribolium castaneum*, and *Prostephanus truncatus*) (Obeng-Ofori and Reichmuth, 1997). The aromatic ether, anisole, elicited a moderately high response in males (0.76, normalized), while the aromatic ester, methyl salicylate, evoked similar responses in both males and females (0.71 and 0.90, normalized respectively). Anisole has been found to be a major component in the sex pheromone of the scarab beetle, *Holotrichia reynaudi* (Ward et al., 2002). Methyl salicylate has been found to be a herbivore-induced plant volatile (HIPV). HIPVs act to attract predatory insects (James and

Price, 2004). Sabinene was found to elicit a similar response in both males and females (0.69 and 1.03, normalized respectively). This compound naturally occurs in the Norway spruce, nutmeg, and the evergreen oak (Shulgin et al., 1967). Two fatty acid derivatives, *n*-undecane and heptanal elicited depolarizations of 1.01 and 0.75, normalized, respectively. Heptanal is found in the blooming linden, while n-undecane is a highly volatile alarm pheromone in ants (Wynne et al., 1991; Haak et al., 1996). The latter compound could serve as an olfactory guide for *A. domesticus* in food source location. Two green leaf volatiles were used throughout this experiment; *trans*-3-hexen-1-ol (control) and *cis*-3-hexen-1-ol (1 and 0.98, normalized). These compounds are typical HIPVs. Triethylamine elicited large depolarizations in both males and females (0.81 and 0.98, normalized, respectively). This scent is derived from the hawthorn plant and bears a strong odor reminiscent of ammonia, in keeping with the fact *A. domesticus* is omnivorous and can feed on decaying plant and animal matter.

Sexual Differences

The compounds that elicited significantly different responses between the sexes were *p*-cresol (p=0.0045; students *t-test*) and 2 methyl butyl benzoate (p=0.0005; students *t*-test) (Fig. 3). Examples of the compounds eliciting EAG responses between males and females are shown in Figs. 4-6. Females typically elicited more robust EAG responses than males with respect to 2-acetylfuran, *p*-cresol, and sabinene, while 2 methyl butyl benzoate elicited a more robust response in males. No difference in the magnitudes of responses, however, was noted for *m*-cresol, methyl salicylate, and tri-ethylamine between the sexes. Shields et al., (2014) (in preparation) did not note differences in the

number and distribution of olfactory sensilla between the sexes of *A. domesticus* and postulate that differences in EAG responses between the sexes may be accounted for by possible differences in the molecular receptive ranges of these sensilla. Differences in the number of sensilla between the sexes were observed, however, for two species of Australian gryllacridids (*Ametrus* sp. 7 and *Bothriogryllacris pinguipes)* (Bland and Rentz, 1994), as well as the cockroach, *Periplaneta americana* and mantid species. In the latter two species, a higher number of olfactory basiconic sensilla were found on male antennae, possibly for the perception of female sex pheromone and the ability to perceive a wide range of host-plant odorants (Chapman, 1982).

Dose-Responses

Dose-response experiments were carried out using two compounds: 2 acetyl furan and *m*-cresol. I found that the antennae of both sexes responded to 2-acetyl furan and *m*-cresol in a dose-dependent manner. Both 2-acetyl furan and *m*-cresol elicited robust responses in both sexes (Figs. 7, 8). The concentrations tested spanned a total of more than 2 log units for each compound. Dose-response curves for both compounds revealed threshold responses for both sexes at 50 µg. For 2-acetyl furan, the normalized EAG values observed at this dose for both males and females were 0.30 and 0.34, normalized, respectively, while at the highest dose tested (i.e., 5000 µg), they were 0.74 and 1.02 normalized, respectively. For *m*-cresol, these values were 0.54 in males and 0.25 in females at 50 µg and 1.02 and 0.70, normalized, respectively, at 5000 µg.

When recording, it is possible to elicit the responses from mechanosensory sensilla on the antennae, rather than from the ORCs housed in olfactory sensilla. To

account for this possibility, responses from blank cartridges (i.e., mineral oil or hexane, alone) were recorded and found to elicit very minimal responses. The threshold response for both acetyl furan and *m*-cresol in males and females was 50µg. A Students *t-test* was carried out to confirm that responses seen to 10µg doses of *m*-cresol and 2 acetyl furan in males and females were not significantly different from responses to blank cartridges, indicating that any activity was due to mechanosensory stimulation and not olfactory perception of the compound. In males, responses to 2 acetyl furan at 10µg were 0.216 normalized, while blank elicited responses of 0.186 normalized ($p=0.73$; students *t-test,* n=6-8); *m*-cresol elicited responses at 10µg of 0.151 normalized, while blanks elicited 0.186 normalized, $p=0.39$ (students *t-test,* n=6-8). In females, responses to 2 acetyl furan at 10µg were 0.169 normalized, while blanks elicited a response of 0.173 normalized ($p=0.95$; students *t-test*, n=5-8); *m*-cresol elicited response at 10µg of 0.140 normalized, while blanks elicited 0.173 normalized ($p=0.68$; students *t-test*, n=5-8).

Conclusions

This study will help us to provide insights into peripheral olfactory processing, especially with respect to orthopterans, where limited work has been carried out with respect to host-plant associated volatile compounds. In the future we plan to continue this project and to investigate plant associated volatiles which are repellent to *A. domesticus.* In addition, this research will yield an increased understanding of how and what olfactory information is processed in the insect nervous system and contribute to the chemical identification of important plant volatiles for insect-plant interactions.

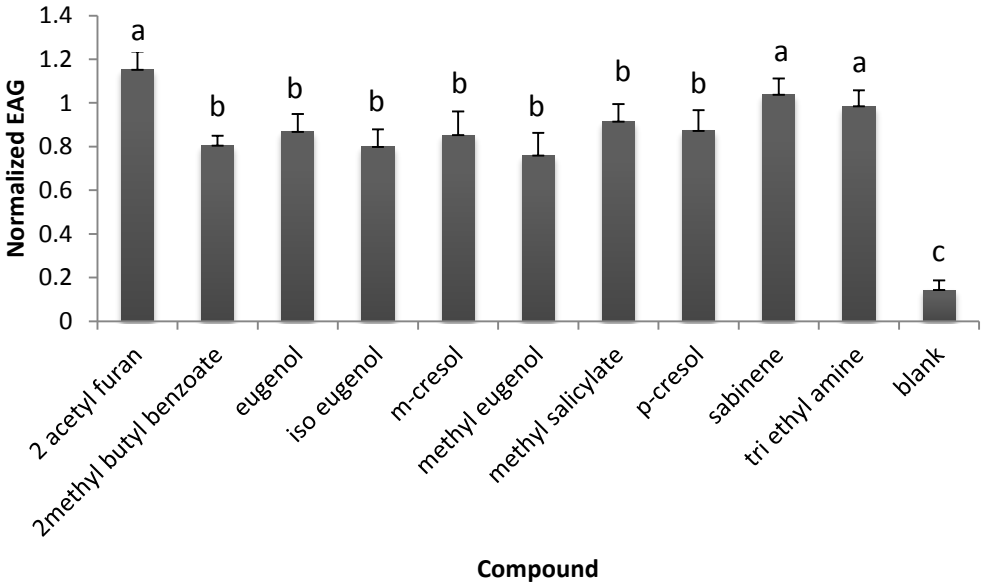


Fig. 1. *Acheta domesticus* female EAG responses to a panel of ecologically relevant volatiles (500µg dose) (n=8-12). Responses have been normalized to the standard stimulus, 500µg of *trans*-3-hexen-1-ol, Means ± 95% C.I.

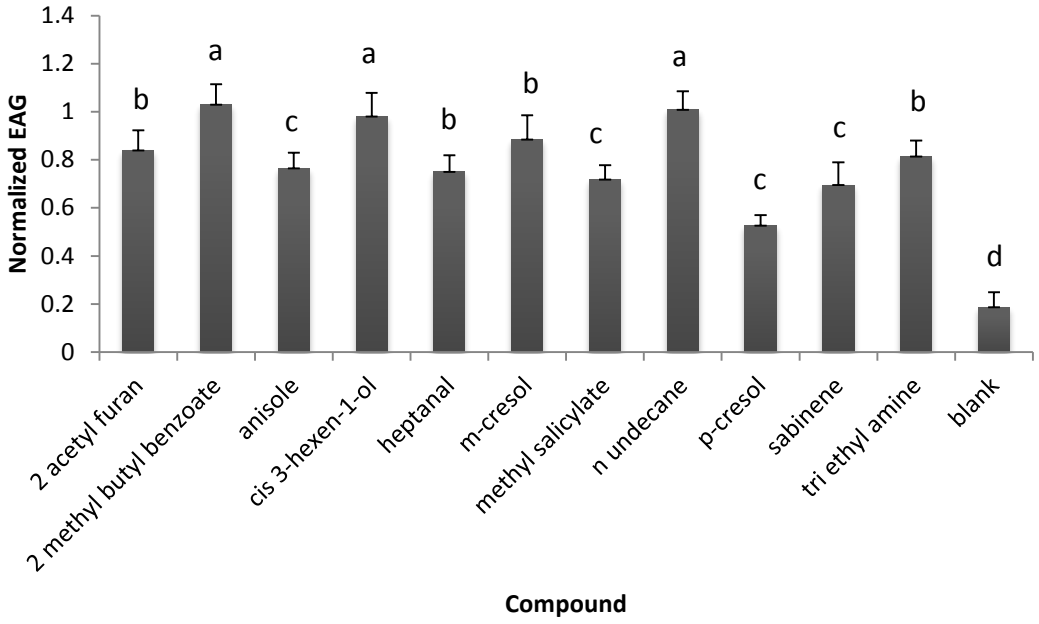


Fig. 2. *Acheta domesticus* male EAG responses to a panel of ecologically relevant volatiles (500µg dose) (n=8-12). Responses have been normalized to the standard stimulus, 500µg of *trans*-3-hexen-1-ol, Means ± 95% C.I.

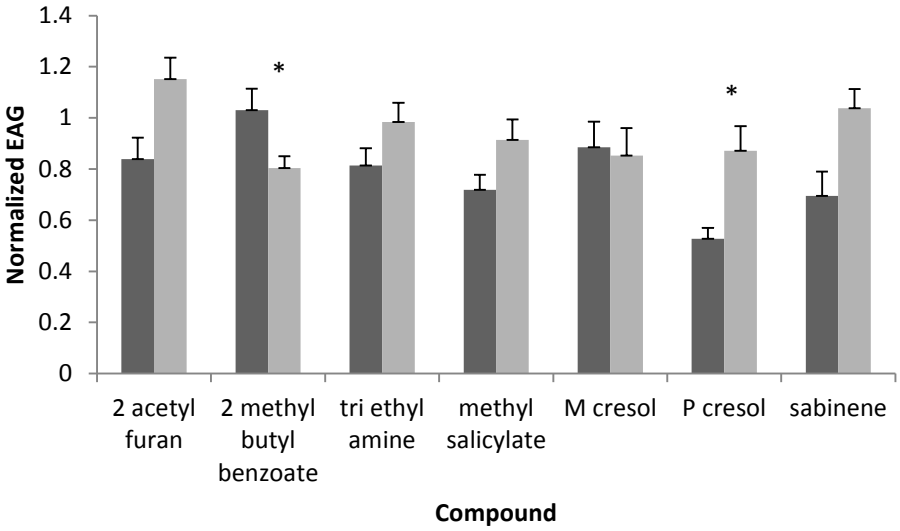


Fig. 3. -Female, Male; Normalized EAG Responses of male and female *Acheta domesticus* to ecologically relevant odorants (500µg dose) (n=8-12). Responses have been normalized to the standard stimulus, 500µg of *trans*-3-hexen-1-ol, Means ± 95% C.I.

* indicates significant difference between sexes according to Students *t-test* with a Bonferroni correction $p<0.007$.

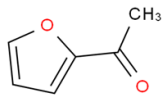
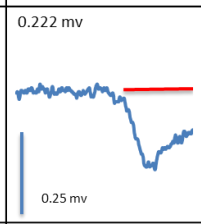
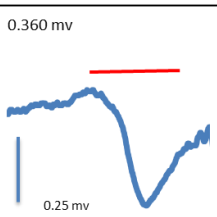
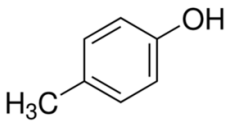
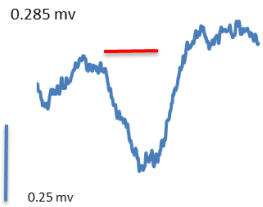
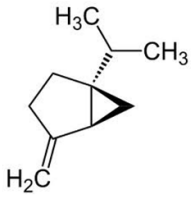
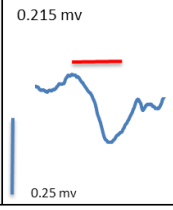
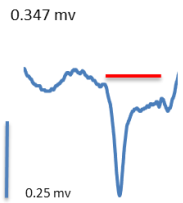
Compound	Male Response	Female Response
2 acetyl furan 		
p-cresol 		
sabinene 		

Fig. 4. Typical electroantennogram responses of male and female *Acheta domesticus* to three volatiles; 2-acetyl furan, *p*-cresol, and sabinene. The horizontal line appearing above each EAG indicates the duration of the stimulus application. Actual non-normalized EAG depolarization values (mV) for each recording is indicated in the top left corner of each recording.

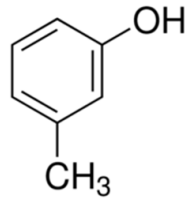
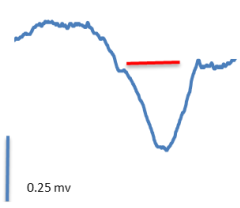
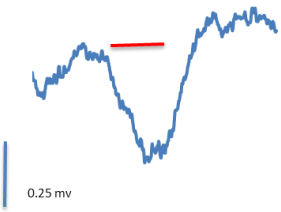
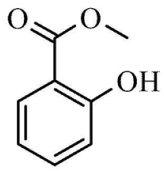
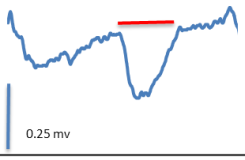
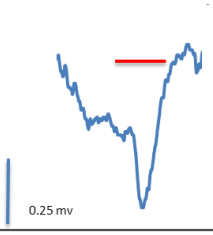
Compound	Male Response	Female Response
m-cresol 	0.264 mv 	0.294 mv 
methyl salicylate 	0.256 mv 	0.276 mv 

Fig. 5. Typical electroantennogram responses of male and female *Acheta domesticus* to two volatiles; *m*-cresol and methyl salicylate. The horizontal line appearing above each EAG indicates the duration of the stimulus application. Actual non-normalized EAG depolarization values (mV) for each recording is indicated in the top left corner of each recording.

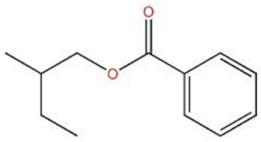
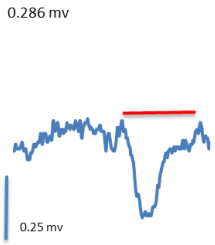
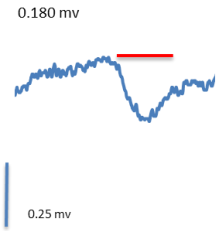
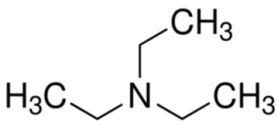
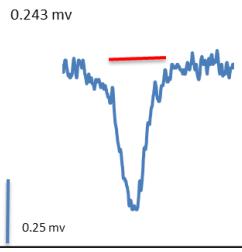
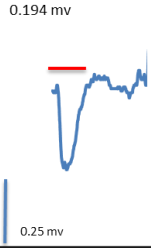
Compound	Male Response	Female Response
2 methylbutylbenzoate 		
tri-ethylamine 		

Fig. 6. Typical electroantennogram responses of male and female *Acheta domesticus* to two volatiles; 2-methylbutylbenzoate and tri ethylamine. The horizontal line appearing above each EAG indicates the duration of the stimulus application. Actual non-normalized EAG depolarization values (mV) for each recording is indicated in the top left corner of each recording.

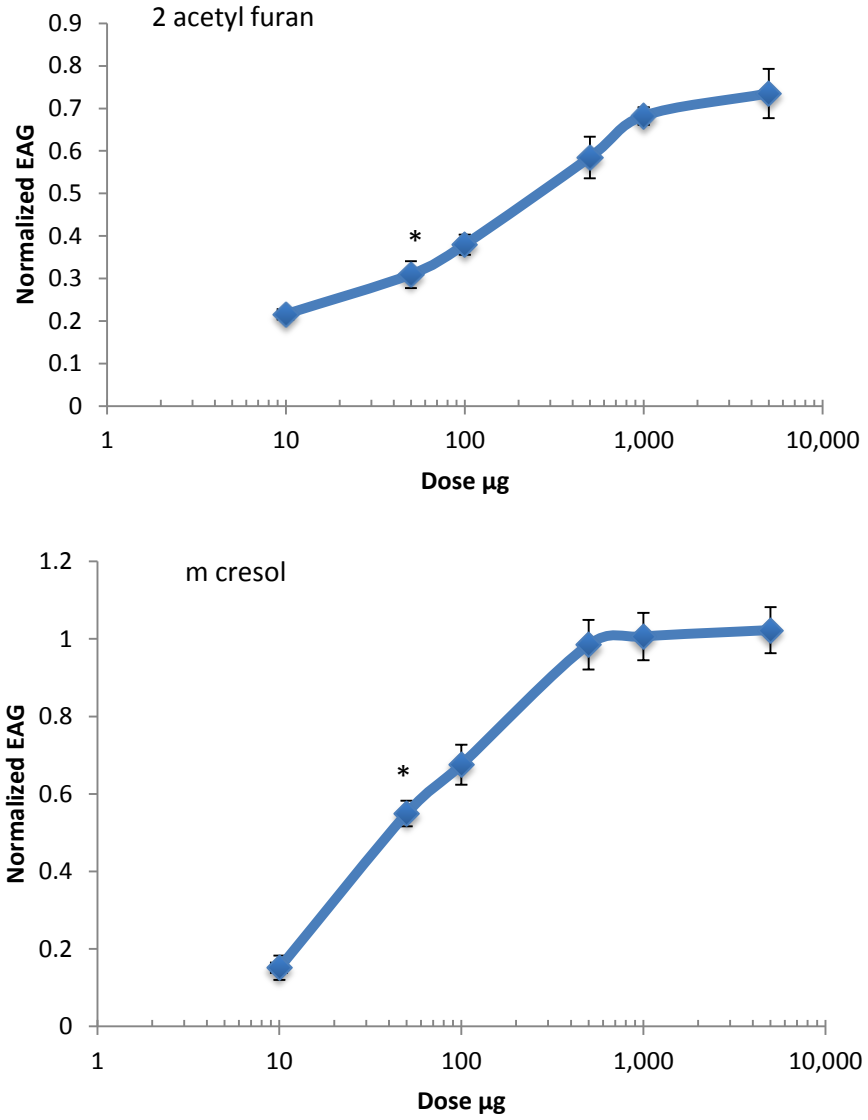


Fig. 7. Male *A. domesticus* EAG dose-responses to 2 acetyl furan and m cresol (mean ± SEM, n=6). The x-axis indicates the amount of compound (i.e., dose) (µg). On the y-axis, EAG responses were normalized against mean EAG responses to 500µg of *trans*-3-hexen-1-ol. * indicates threshold response; $p<0.05$.

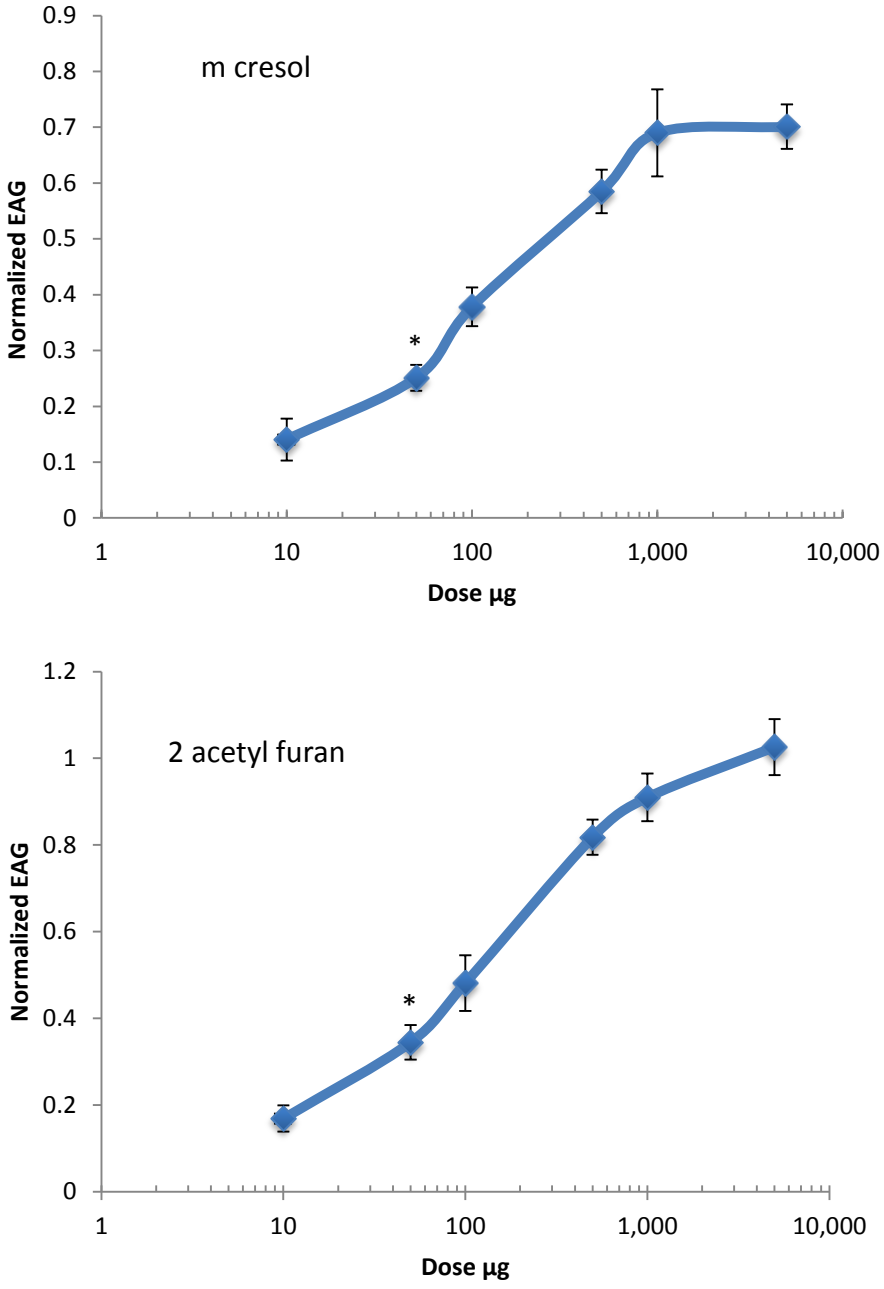


Fig. 8. Female *A. domesticus* EAG dose-responses to 2 acetyl furan and *m*-cresol (mean ± SEM, n=6). The x-axis indicates the amount of compound (i.e., dose) (µg). On the y-axis, EAG responses were normalized against mean EAG responses to 500µg of *trans*-3-hexen-1-ol. * indicates threshold response; $p<0.05$.

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Curriculum Vitae

MARY LATIMER

[redacted]

GRADUATE EDUCATION
Towson University
Degree: Master of Science
Major: Biology
Current GPA: 3.94
Anticipated Graduation Date: 5/15/14

UNDERGRADUATE EDUCATION
Georgia State University
Degree: Bachelor of Science
Majors: Molecular Biology
Minor: Chemistry
GPA: 3.53

WORK EXPERIENCE

- **Graduate Teaching Assistant** – Department of Biological Sciences, 8000 York Rd, Towson University, Towson MD, 21252. (8/2013 – Present)
    - Teach three, two 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 reports and grading of reports to monitor student performance

- **Graduate Assistant for the Office of Undergraduate Research**- Department of Biological Sciences, 8000 York Rd, Towson University, Towson MD, 21252. (8/2012-5/2013)
    - Assisted Director in all duties related to disbursement of research, travel, and small project grants given to undergraduate students each semester
    - Co-coordinated the annual "Graduate and Undergraduate Student Research and Scholarship Expo" held on Towson's campus highlighting completed and ongoing research in all disciplines

- **Physical Sciences Technical Specialist** – Anne Arundel Community College, Anne Arundel, MD (3/2011 - 8/2012)

- Handled ordering, maintenance, and security protocols associated with the general and organic chemistry laboratories
- Guided students in using specialized equipment including: Nuclear Magnetic Resonance Spectrometry, Atomic Absorption Spectrometry, and Gas Chromatography
- Performed maintenance on all equipment used in the general and organic chemistry laboratories

- **Student Research Assistant** – Department of Biological Sciences, P.O. Box 3965, Georgia State University, Atlanta GA, 30302. (1/2009 – 7/2009)

    - Assisted graduate students in performing; solid phase protein synthesis, high performance liquid chromatography, and radioactive assays
    - Assisted in general lab maintenance and attended all laboratory meetings
    - Attended local scientific meetings to discuss ongoing research in the laboratory

- **Chemistry Laboratory Teaching Assistant**- Department of Biological Sciences, P.O. Box 3965, Georgia State University, Atlanta GA, 30302. (8/2008-12/2008)

    - Reviewed and prepared chemistry lab teaching plans
    - Assisted in lecture and student teaching
    - Assisted in maintenance and inventory of chemistry laboratory and equipment

RESEARCH PROJECTS

- 8/2012 – Present – Electrophysiological examination of the response of the antennae of the common house cricket, *A. domesticus*, to plant associated volatiles
- 8/2012-Present- Using a dual choice behavioral assay to determine biologically relevant odorants to the common house cricket, *A. domesticus*.
- 5/2010-8/2010 – Investigation of Pyridopyrimidine molecules as inhibitors of bacterial quorum sensing
- 1/2009-7/2009- Using histone modification to investigate dual drug therapies in treating prostate cancer

PUBLICATIONS

Neslund MC, Baker TC, and Shields VDC (2014) Comparison of Male and Female Electroantennogram Responses to Plant−Associated Volatiles in the Common House Cricket, *Acheta domesticus*. Manuscript in Preparation.

TECHNICAL SKILLS

Software - Microsoft office, NCSS 2007, "R"

ORAL/POSTER PRESENTATIONS

- Towson University 14th Annual Student Research and Scholarship Expo, Towson, Maryland – Oral and Poster Presentation (04/2014)
- Society of Integrative and Comparative Biology (SICB) 2014, Austin, TX. (1/2014)
- Towson University 13th Annual Student Research Scholarship Expo, Towson, Maryland (4/2013)
- Georgia State University Annual Student Research Expo, Atlanta, GA. (8/2010)

AWARDS, GRANTS, and HONORS

- Charlotte Magnum Student Support Fund - $140 for registration support to SICB 2014 –(1/2014)
- Towson University Graduate Student Association Award – $500 (10/2012)(01/2013)
- Towson University School of Emerging Technology – Award presented to major professor, VDC Shields, funded ongoing research and a summer research stipend – (6/2013-8/2013)
- HOPE Scholarship– Awarded all tuition and fees (8/2006-5/2007)(8/2008-5/2010)
- Molecular Basis of Disease Area of Focus– Summer Stipend (5/2009-7/2009)