Journal of Insect Physiology 117 (2019) 103905

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Labial and maxillary palp recordings of the Asian longhorned beetle, *Anoplophora glabripennis*, reveal olfactory and hygroreceptive capabilities

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ARTICLE INFO

Keywords:
Asian longhorned beetle
Anoplophora glabripennis
Cerambycidae
Olfaction
Palps
Electrophysiology
Electropalpogram
EPG
Single sensillum recording
SSR

ABSTRACT

Electrophysiological recordings from the labial and maxillary palps of the Asian longhorned beetle, *Anoplophora glabripennis,* revealed their ability to detect several volatile chemicals, including water vapor and acetic acid. The results indicate that these appendages may play a large role in this beetle's assessment of its immediate environment. *A. glabripennis* is a highly destructive, invasive pest that feeds preferentially on maple — but accepts many other tree species — in North America, warranting USDA quarantine zones and an eradication program. While control and sampling techniques are being developed for this insect, a better understanding of its sensory capabilities is helpful. Electropalpograms (EPGs) revealed that both the maxillary and labial palps are highly sensitive to changes in humidity, indicating the presence of hygroreceptors and the likely important role of humidity in such things as feeding and finding water or oviposition sites. Strong EPG responses to a narrow set of volatile chemicals indicate that olfactory sensory neurons (OSNs) on the palps may be tuned to a small number of volatile compounds. The types of odorant molecules eliciting responses indicate that there are likely both odorant receptors (ORs) as well as ionotropic receptors (IRs) expressed on the OSNs, enabling palp OSNs to be able to respond to acids and aldehydes such as acetic acid and butyraldehyde. There were no significant EPG responses to this species' trail-sex pheromone components, which may indicate that the trail pheromone is primarily perceived via gustatory receptors contacting the substrate. These results indicate that the palps have a role in the beetle's assessment of its immediate environment underfoot, and that the sampling of surface odors and humidity via mouth parts may be important to this species' success.

1. Introduction.

The maxillary and labial palps are insect mouthparts that are usually associated with contact chemoreception, i.e., gustation, and are equipped with sensilla containing gustatory chemo-receptive sensory neurons. Insect palps may also be used for detecting volatile chemicals when they are equipped with sensilla containing olfactory sensory neurons (OSNs) such as are seen in the maxillary palps of malaria mosquitoes (*c.f.* George et al. 2011). The axons of OSNs originating from sensilla on insect palps terminate and arborize with second-order neurons in the antennal lobe, but in a more ventral (posterior) region than do antennal OSNs (Kent et al., 1986, Dippel et al., 2016). Most notably, dipterans are well known (Ayer and Carlson, 1992) and studied (de Bruyne et al., 1999; Syed and Leal, 2007) for utilizing their maxillary palps for olfaction. Lepidopterans are also known for some species' olfactory utilization of palps (Kent et al., 1986), as are some orthopterans (Blaney, 1973). Coleopteran palpal olfaction has likewise not been overlooked by researchers. Morphological (Giglio et al., 2013) and genetic methods (Dippel et al., 2016) have been primarily used to examine coleopteran palps for their olfactory ability.

However, there have been a very few electrophysiological studies of beetle olfaction via mouthparts. Eilers et al. (2012) discovered, using electropalpograms (EPGs) that the labial palps of scarabid beetle larvae are olfactory as well as water-sensing. In electrophysiological and

Abbreviations: EPG, electropalpogram; SSR, single sensillum recording; OR, odorant receptor; IR, ionotropic receptor; OSN, olfactory sensory neuron

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https://doi.org/10.1016/j.jinsphys.2019.103905
Received 6 March 2019; Received in revised form 20 June 2019; Accepted 21 June 2019
Available online 22 June 2019

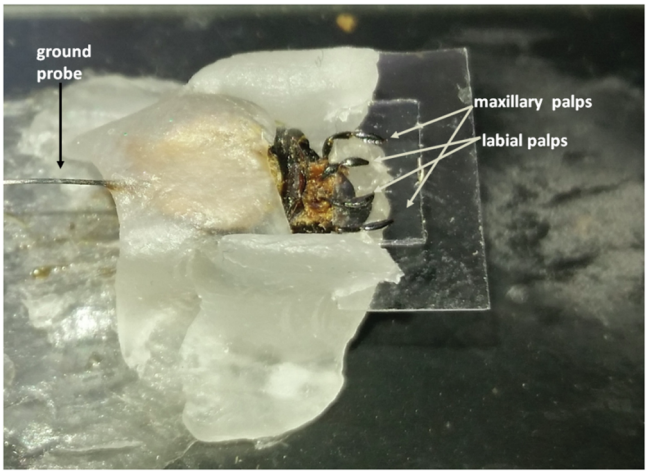


Fig. 1. Palps prepared for Electropalpogram/Single sensillum (EPG/SSR) recordings. The head is removed from the body and held in place using dental wax with the ventral surface of the head facing upwards. The maxillary and labial palps are affixed to a glass cover slip using double-sided transfer tape. To the left, the grounding electrode is protruding from the neck opening, which is sealed with wax.

behavioral experiments, the palps of adults of the carabid beetle, *Siagona europaea,* were found by Talarico et al. (2010), to respond to olfactory stimuli. With their impressive antennae, the Asian longhorned beetle (ALB), *Anoplophora glabripennis* and other cerambycids have attracted electrophysiologial attention at the antennal level (Crook et al., 2014, Toshova et al., 2016, Fan et al., 2007, Hall et al., 2006, Liendo et al., 2005, Wei et al., 2018), but to date there has been no study published in searchable English-language journal databases that has reported electrophysiological examination of palp olfaction in the Cerambycidae. Therefore, the research reported here addresses some relatively uncharted territory for coleopteran, and specifically cerambycid, experiments: interrogating the mouthparts for their ability to detect airborne chemicals.

Individuals of another cerambycid, *Monochamus alternatus,* have been observed palpating around oviposition sites (Anbutsu and Togashi, 1997, 2000). This study focused on the U.S.-invasive cerambycid species, *A. glabripennis*. Graves et al., 2016 suggested that the maxillary and labial palps of *A. glabripennis* males were important for detecting this species' female-deposited trail-sex pheromone blend. Those experiments showed that the trails could not be successfully followed by males without directly contacting them, suggesting that only contact chemoreception might be used for following these pheromone trails. However SEM images (Meng, 2014) seemed to indicate the sensilla at the tips of the palps were located in a concave-shaped

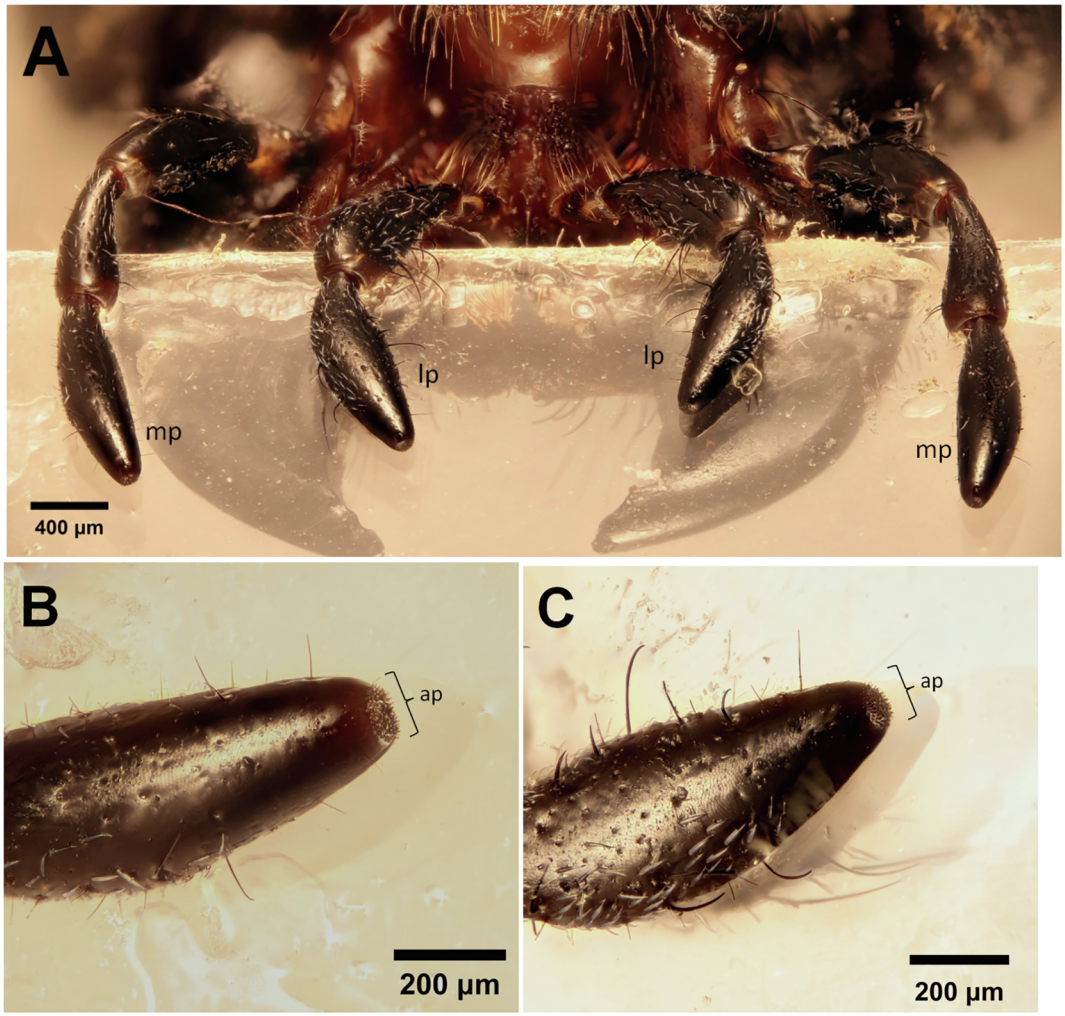


Fig. 2. A. The *A. glabripennis* palps showing both the labial (lp) and maxillary (mp) palps. Higher magnification photographs of the maxillary palp (B) and the labial palp (C) with the apical pits (ap) of the palps in the flexed-out position. The array of pit sensilla are thrust out as the "pit" is now more of a dome during this outward flexing of the apical pit membrane. (Photographs in this figure are by Carolyn Trietsch of the Andrew Deans Laboratory, Pennsylvania State University.) Video of apical pit flexing from concave to convex recorded by Loyal Hall can be viewed at https://youtu.be/3RGu4_aClRo.

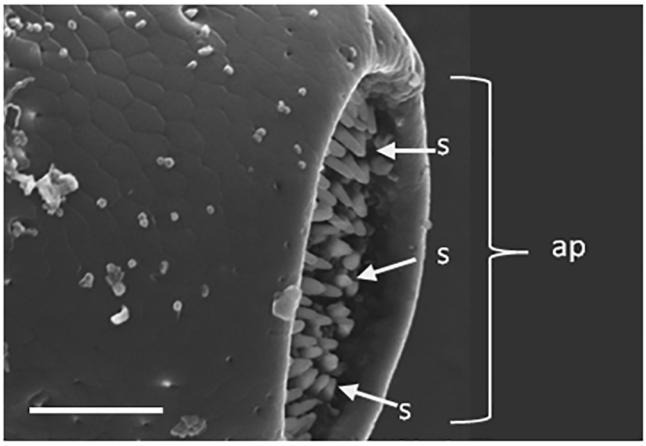


Fig. 3. SEM image of the tip of a female ALB maxillary palp, showing the presumed concave architecture and many sensilla of the apical pit (ap) surface. Scale bar = 5 µm From P. S. Meng (2014), Master's Thesis, Penn State University.

apical pit and thus unable to make direct contact with a substrate and would likely be olfactory.

Such studies raised a general question about whether the palps of cerambycids had the ability to detect airborne compounds vs. using them only for contact chemoreception. It has been observed that *A. glabripennis* palps are constantly in motion and directly touching surfaces as the beetle walks along (Li et al., 1999). Suggestions have been made that the trail-sex pheromone as detected using contact chemoreception results in gravid females being able to space out their eggs to prevent larval cannibalism (Graves et al., 2016). But might an olfactory capability afford a greater utility for the palps, for instance in allowing favorable feeding or oviposition sites on a tree to be detected from greater distances than would be possible via contact chemoreception?

2. Materials and methods

2.1. Preparation

Beetles were reared on a pourable modification (Keena, 2005) of a diet designed for *Enaphalodes rufulus* at 27.5 °C for 90 days, chilled at 10 °C for 90 days, then returned to 27.5 °C during which time the larvae were allowed to develop until pupation. At this time, the pupae were transferred to 50 ml Falcon centrifuge tubes and incubated at 27.5 °C until adult eclosion. Adults were fed red maple (*Acer rubrum*) twigs until preparation for electrophysiological study. Only virgin adults, about 25 days old (± 5 days) which had recently had their maturation feeding were used for the study.

To prepare individuals for study, the palps were required to be absolutely immobile. In order to keep the tissue functioning longer, the palps were not removed from the head. Rather, the entire head was removed from the body with a razor blade and the antennae removed with scissors. The head was then held in place with dental wax on a glass slide, leaving the mouthparts exposed (Fig. 1). A tungsten reference probe was inserted through the open neck cavity into the neural tissue. The opening was then closed with wax to prevent dehydration. A cover slip was cut to fit the size of the head capsule and mouthparts then covered with a piece of 3M 9474LE 300LSE super-strong double-sided adhesive transfer tape (3M, USA). This cover slip was gently interposed between the mandibles and the palps with the lateral edges embedded in the dental wax that held the head. Forceps were then used to maneuver the palps and affix them in the adhesive.

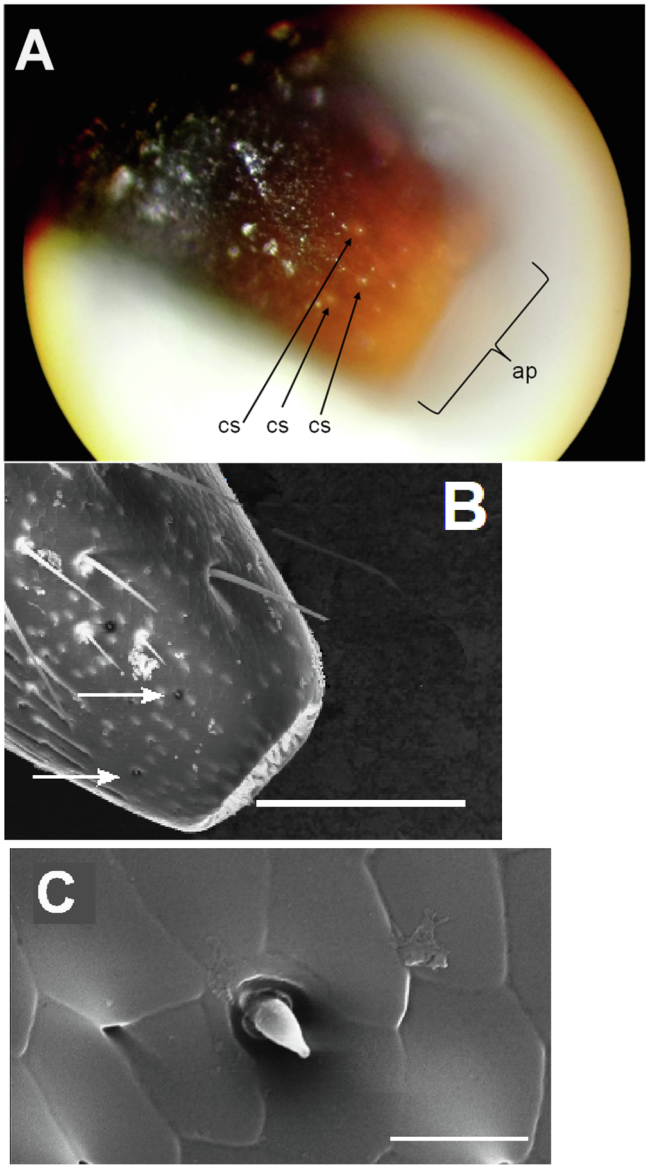


Fig. 4. A. Light microscope image of the maxillary palp. Coeloconic sensilla (cs) and apical pit (ap) are indicated. B. SEM image of the tip of a female ALB maxillary palp, arrows indicate coeloconic sensilla. Scale bar = 10 µm. C. SEM high magnification image of a coeloconic sensillum on the maxillary palp. Scale bar = 1 µm. (B and C are from P. S. Meng (2014), Master's Thesis, Pennsylvania State University.)

2.2. Palp structure

The maxillary and labial palps of male and female *A. glabripennis* consist of four and three palpomeres, respectively. Each palp segment has thick cuticle sparsely populated with setae and possible chemo- and mechanoreceptive sensilla (Fig. 2). The terminal segments of both pairs of palps terminate with an apical pit festooned with possible chemoreceptive sensilla (Figs. 2 and 3).

An unexpected and interesting observation made was a flexing of the apical pits on the tips of the maxillary and labial palps. It had been assumed from scanning electron microscope (SEM) images (Meng, 2014) that the palp tip consisted of a concave apical pit harboring stubby sensilla (Fig. 3), but it appears that this fixing of the palp in that concave position was an artifact of preparation for SEM imaging. The

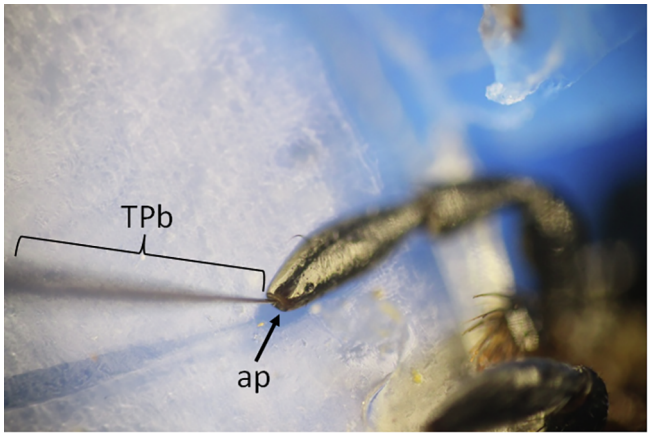


Fig. 5. Tungsten probe (TPb) placed against the apical pit of the palp (ap) for EPG recording.

apical membranes of living palp tips containing the sensilla were observed to regularly flex from concave to convex, with the sensilla bulging out in a shallow dome into the environment (Fig. 2). Videos of this movement can be found in the supplementary materials or viewed at https://youtu.be/3RGu4_aClRo.

2.3. Electropalpograms

For electropalpogram recordings (EPGs), tungsten electrodes were prepared by electrolytically sharpening them using a saturated KNO_2 solution at 10 V. The sharpened tungsten wire was placed into contact with the membrane comprising the apical pit of either the maxillary or labial palp (Fig. 5) using a Narishige hydraulic micromanipulator, completing the electrical circuit and allowing the collective neural activity of the palp to be recorded. A custom high-impedance DC-coupled preamplifier and 16 bit analog-to-digital converter with a conversion rate of 46,875 Sa/s and voltage range of −1 to +1 V was used to make recordings. These data were then re-sampled at 10 KSa/s and stored for later analysis. The difference between the voltage at the beginning of an EPG response and the lowest or highest peak was then measured (Fig. 6) and entered into a spreadsheet along with the study subject identifier, sex, palp type, and odorant.

The odorants used in this study (Table 1) were diluted in hexane in 10-fold steps to different concentrations such that odor cartridges contained loadings of either 1 µg, 10 µg, or 100 µg of an odorant when aliquots of 10 µl of each odorant dilution were dispensed onto a filter paper strip (Whatman; 0.2 × 1.5 cm). Each strip was inserted into a 15 cm Pasteur pipette to create each of the odor cartridges. A constant airflow of charcoal-purified, humidified air was passed across the palps through a glass tube (10 mm diameter) during the experiments. Odorants were delivered into this constant air stream via the Pasteur pipette whose tip was inserted through a small hole in the glass tube, 11 cm away from its end. A stimulus flow controller (Syntech; Germany) pulsed a 40 ml/s air stream through the cartridge for 0.3 s, effectively delivering a puff of volatiles from the odor cartridge into the air stream and onto the antenna. Odor cartridges were kept chilled (-15C) when not in use, used in batches of 8–10, allowed to attain room temperature before use, and then returned to cold storage. Cartridges were remade every 30 puffs for lower volatility odorants and every 3 puffs for highly volatile odorants. All odors were tested at 100 µg with the exception of (3*E*,6*E*)-α-farnesene, due to supply issues, and the other pheromone components which were tested at 10 and 100 µg to determine if there was a dose-dependent response.

When conducting EPG measurements of the full panel of odorants the order of odorants used was constant and thus measurements of a control odorant, geraniol, were taken at the beginning, middle, and end of the series for every individual palp in order to measure the decay/change of the tissue/connection for each individual palp. (Even if the response to geraniol is not different from a blank, the EPG is still a real response to a stimulus and thus can be used to ascertain changes in connection quality.) These three measurements were then used to adjust the EPG measurements of the odorants measured, according to their order of use in the panel, to account for this decay for each individual palp using the equation $X_{adj} = X(G_{mean}/G_{close})$ where X is the measurement of the EPG response to the odorant multiplied by the ratio of the mean of the three geraniol responses to the temporally closest of the three geraniol measurements taken to X.

2.4. Single sensillum recordings

Single sensillum recordings (SSR) were conducted similarly to EPGs except for the recording electrode placement. The electrode was placed against the base of one of the coeloconic sensilla that sparsely populate the outer cuticle along the last segment of the maxillary palps (Fig. 4) which were accessible when the palps were positioned as shown in Fig. 2A, after which the action potentials of olfactory sensory neurons (OSNs) could be recorded within the sensillum. The output from the electrode could also be configured to now simultaneously record the DC potential from within that sensillum along with SSR action potentials by adjusting the highpass cutoff to 500 Hz. In this configuration, the recordings of both the DC hyper/depolarizations and the AC action potential spike trains happening within the sensillum could be compared (Fig. 15). Due to the capriciousness of these connections, only acetic acid, butyraldehyde, water, trail pheromone, and blank were tested at 100 µl.

2.5. Sensitivity to moisture levels

Three dosages of water, 1 µl, 10 µl and 100 µl, were loaded onto the filter papers of three different odor cartridges, and no water at all was in a fourth, blank, cartridge. Injections were done using plain tubing in the puffed air injection line, with no humidification of the puffed air injected through the odor cartridges into the constant, humidified airstream running over the palps using the methods described in Section 2.3. The results of these tests indicated a need to temper the effects of

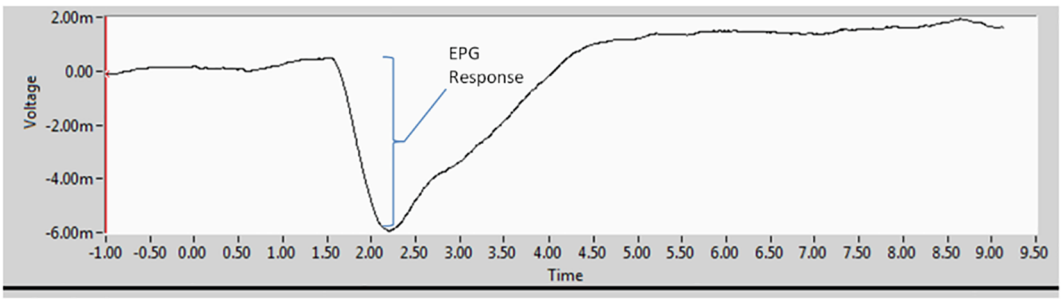


Fig. 6. A hyperpolarizing EPG response from a male labial palp in response to a puff containing 100 µg of geraniol.

Table 1
Odorants used in this study, along with their commercial sources and purities. (Purities for the two *A.glabripennis* male-produced pheromone components were not provided by Bedoukian.)

Odorant	Supplier	Purity (%)
citronellal	Acros Organics B.V.B.A. (Belgium)	93
4-(*n*-heptyloxy) butan-1-ol	Bedoukian Research Inc. (Danbury, CT, USA)	
4-(*n*-heptyloxy) butanal	Bedoukian Research Inc. (Danbury, CT, USA)	
acetic acid	J.T. Baker, Inc. (Phillipsburg, NJ, USA)	100
(3*E*,6*E*)-α-farnesene	Jocelyn Millar Laboratory, University of CA, Riverside	86
trail pheromone, whole blend	Kelli Hoover Laboratory, University of PA	97
trail pheromone, major components	Kelli Hoover Laboratory, University of PA	97
trail pheromone, minor components	Kelli Hoover Laboratory, University of PA	97
isovaleric acid	Sigma-Aldrich Corporation (St. Louis, MO, USA)	97
benzoic acid	Sigma-Aldrich Corporation (St. Louis, MO, USA)	97
Z-2-hexenal	Sigma-Aldrich Corporation (St. Louis, MO, USA)	97
butyraldehyde	Sigma-Aldrich Corporation (St. Louis, MO, USA)	98
1-4-diaminobutane	Sigma-Aldrich Corporation (St. Louis, MO, USA)	97
β-caryophellene	Sigma-Aldrich Corporation (St. Louis, MO, USA)	98
Z-3-hexen-1-ol	Sigma-Aldrich Corporation (St. Louis, MO, USA)	98
linalool	Sigma-Aldrich Corporation (St. Louis, MO, USA)	99
α -terpineol	Sigma-Aldrich Corporation (St. Louis, MO, USA)	96
geraniol	Tokyo Chemical Industry Co., Ltd. (Japan)	96
eugenol	Tokyo Chemical Industry Co., Ltd. (Japan)	98

water vapor fluctuation detection when testing for odorant detection (see results Section 3.1).

For this reason we designed an apparatus to add water vapor to every puff of air that was injected into and through the odor cartridge. For conducting the odor-sensitivity experiments, both EPGs and SSRs, puffs where humidified by first being passed through a 15 ml centrifuge tube containing a cotton wick saturated with distilled water and suspended so as not to impede airflow (Fig. 7). The tubing was held in place and made air-tight with Sugru (FormFormForm, UK), a rubberized, self-setting putty. This humidifier cylinder was attached to, and positioned in, the air-puff injection line immediately upstream of the odor cartridge. For non-humidified, ambient humidity puffs, the humidifier cylinder was swapped out with an equal length of tubing.

2.6. Statistics

Unless otherwise specified, statistical comparisons were conducted by first normalizing data either via $\ln(X+|L|)$, where L is the lowest value for the variable being tested multiplied by 1.33 (to move all values above zero) or $3\sqrt{X}$, using whichever method gave the most normalized result. Once a data set was normalized, a General Linear Model (GLM) test followed by a post hoc test was conducted. A Tukey's test was used to compare between treatments (odorants) and a Dunnett's test was used to compare treatments to a control (blank) ($p \leq 0.05$).

3. Results

3.1. Electropalpograms, water vapor sensing

For both sexes, labial and maxillary palps were sensitive to changes in the amount of water vapor present in the constant airstream. EPGs of the labial and maxillary palps in response to various changes in moisture content resulted in altered response levels of the neurons in the palps in a dose-responsive manner, N = 9 per palp type per sex. Injections of drier air caused hyperpolarization whereas injections of more humid air caused depolarization (Fig. 8).

The drier or wetter the injection, the greater was the hyperpolarization or depolarization, respectively (Fig. 9). There was a difference in responsiveness to moisture-level changes between maxillary and labial palps, with labial palps responding to variations in moisture content in a more pronounced fashion than maxillary palps (Fig. 9).

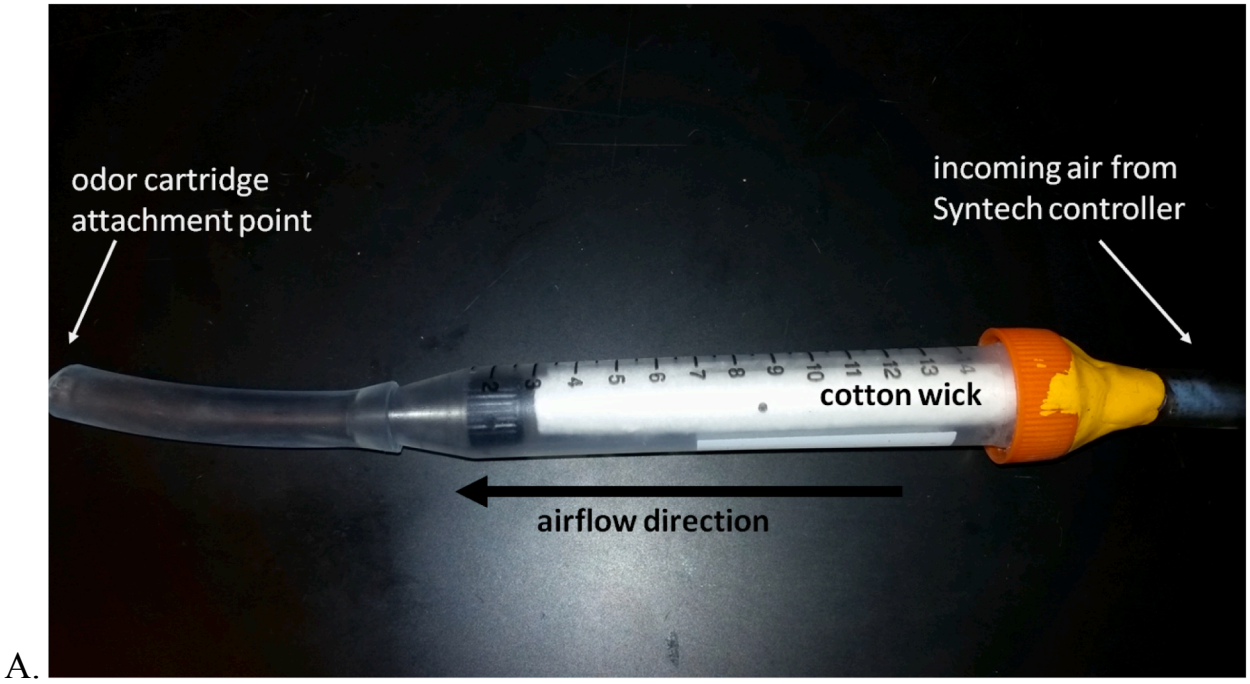
The removal of this confounding response to water vapor reduction is demonstrated by comparing EPGs with and without this humidifying apparatus. The change in water vapor produced some complex combinatorial effects in EPG responses (Fig. 10). Puffs from cartridges containing geraniol, water, or empty filter paper (blanks) – with the puffing airstream being delivered either by passing through the humidifying apparatus or an equal length of plain tubing – were injected into the humidified constant airstream flowing over the palps. An F-test showed that the variances between humidified and un-humidified puffs were unequal in many cases and thus a Wilcoxon signed rank test was used to show differences in EPG magnitude between the two types of puffs for each odorant. When we puffed water, geraniol, or blanks through the odor cartridge into the constant humidified airstream directed at labial or maxillary palps of either sex, we recorded greater hyperpolarized (increased negative mV) EPGs when we used dry, unhumidified air than when we used humidified air. Importantly, a decrease in moisture passing over the palps from drier, ambient air puffed through the cartridges yielded larger variations in responses than when humidified air was puffed through the same cartridges.

3.2. Electropalpograms, odor-sensing

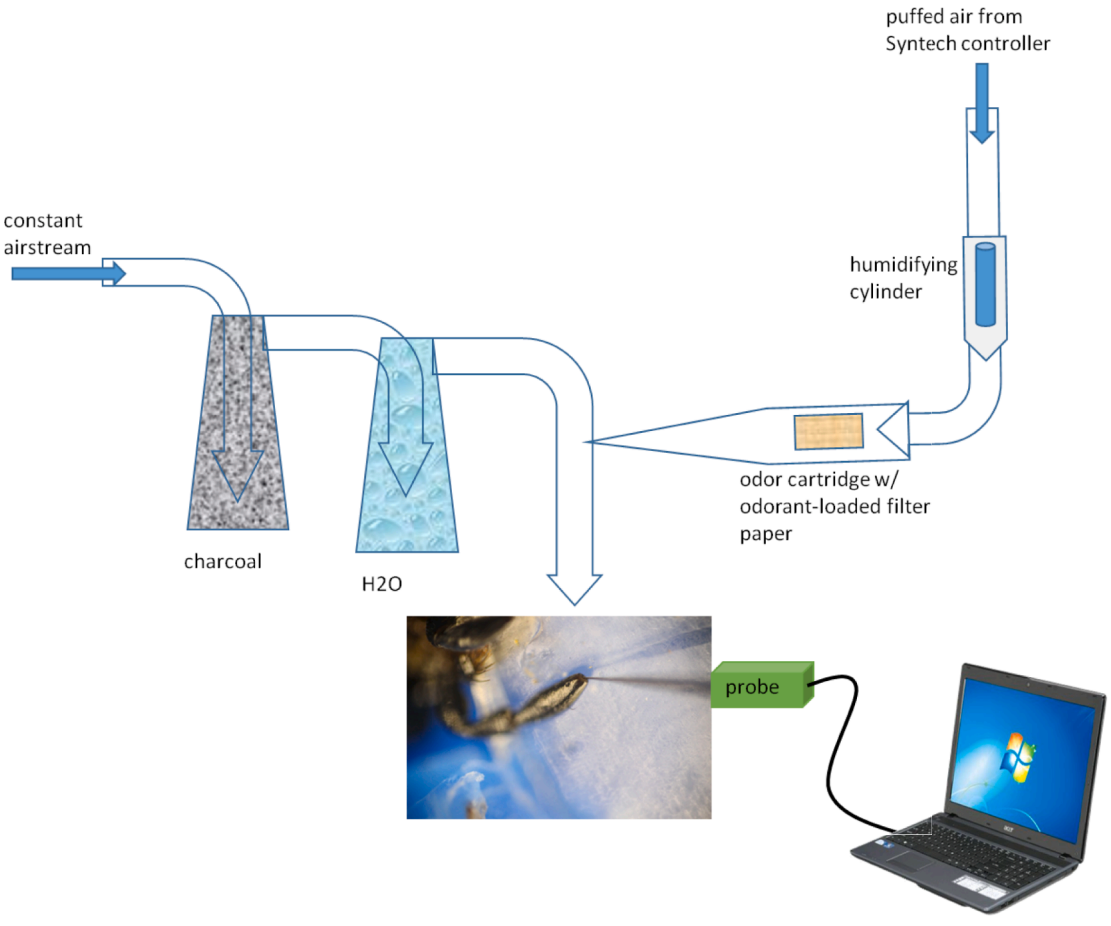
The above EPG results indicate that the maxillary and labial palps of both sexes are able to respond to increases or decreases in the abundance of water molecules. In the experiments presented below, a wide array of odorants were tested, with any changes in moisture levels from the puffed airstream through the odor cartridges tempered through the use of the humidifying apparatus on the puffed-air stream through the odor cartridge that exited into the humidified air constantly flowing over the palps. Several of the odorants produced EPG responses that were significantly different from a blank, N = 11. These results indicate an olfactory ability by the neurons in the labial or maxillary palps to detect certain odorants.

We first compared the amplitudes of EPG responses occurring among all the odorants. For both palp types and both sexes, acetic acid and butyraldehyde elicited the strongest EPG depolarization responses, followed by the depolarization response amplitude to water. All the other odorants tested, including the blank, produced EPGs that were hyperpolarizations and were significantly different from acetic acid, butyraldehyde, and water (Fig. 11 below).

Acetic acid and butyraldehyde caused a tonic depolarization in labial and maxillary palps of both sexes. The initial response was usually a small, brief hyperpolarization that was followed by, or often seemingly masked by, the large tonic depolarization that followed and lasted for sometimes 10 or more seconds (Fig. 12). For the EPG



A.



B.

Fig. 7. Humidifying apparatus for injection line and its placement in the setup.

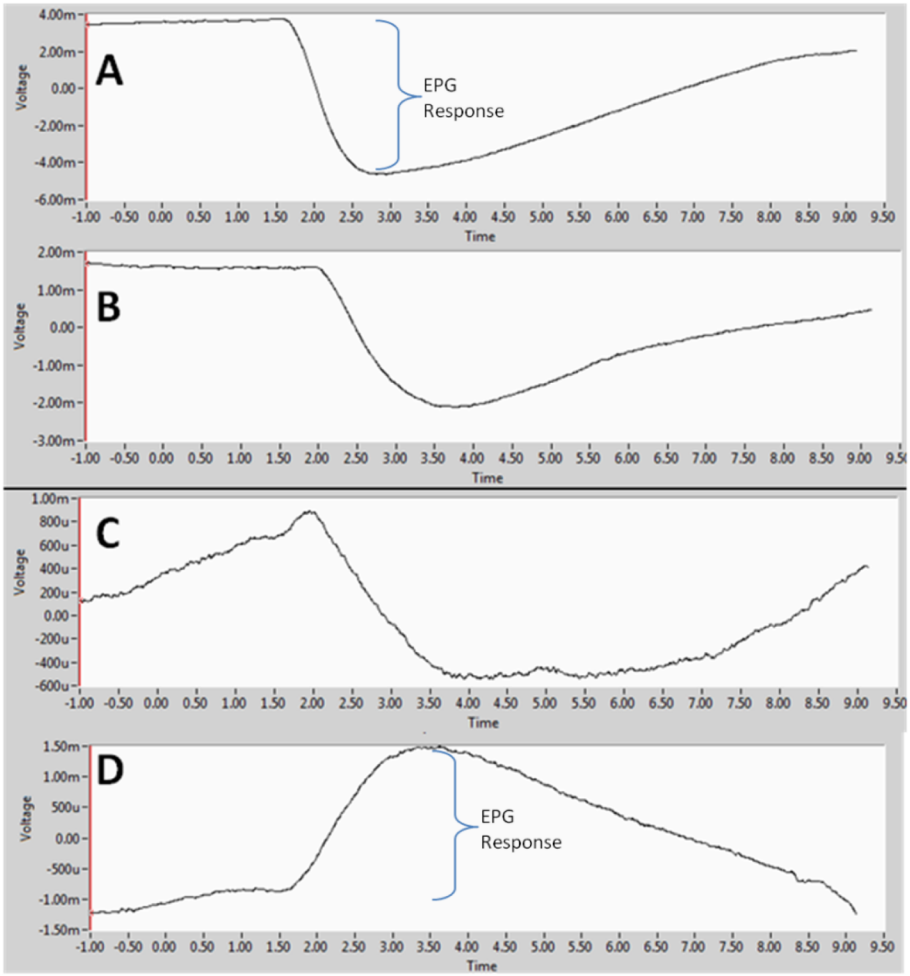


Fig. 8. Series of EPGs from a single male maxillary palp bathed in a humidified airstream to a succession of puffs using ambient, un-humidified air to introduce dosages of water on filter paper of 0 μg, 1 μg, 10 μg, or 100 μg (A, B, C, and D, respectively). With increasing amounts of water in the puffs, the responses changed from hyperpolarizations (A, B, C) to depolarizations (D).

analyses, only the initial, peak level of the tonic depolarization was used for an amplitude measurement.

The hyperpolarizations measured in response to most of the odorants tested seemed of such a different kind of response and of much smaller variances that we tested only the hyperpolarizations to find if any of them were different (more negative) from the blank response (Fig. 13).

Labial palps responded with hyperpolarizing EPGs significantly different from a blank in response to a greater variety of odorants than did maxillary palps, including several plant compounds (benzoic acid, citronellal, eugenol, linalool, α-terpeniol, *Z*-3hexen-1-ol, *β*-caryophellene, (*E*)-2-hexenal, and (3*E,6E*)-α-farnesene, which is also a possible minor sex-aggregation pheromone component (Crook et al., 2014). They also responded to 1,4-diaminobutane.

We found that maxillary palps responded with significant EPGs to the volatiles of only a few odorants, namely 1,4-diaminobutane, linalool, *Z*-2-hexenal, and *Z*-3-hexen-1-ol.

There were some sexual differences in EPG responses. The maxillary and labial palps of males responded to a greater variety of odorants than did female palps (Fig. 13). There were also differences in the magnitude of hyperpolarizations in EPGs (Fig. 14), being greatest in male labial palps, intermediate in maxillary palps of either sex, and lowest in female labial palps. There were no significant differences in the magnitudes of the depolarizations between palp types or sexes.

3.3. Single sensillum recordings

Of numerous attempts, single sensillum recordings (SSRs) were successfully conducted on twelve coeloconic sensilla on the maxillary palps, four sensilla on males and eight on females (Table 2). These sensilla were located along the side of the terminal palp segment, slightly proximal from the palp's terminal apical pit (Fig. 4) and accessible when positioned as seen in Fig. 2A. SSRs were attempted repeatedly on the sensilla located within the apical pit of the palps, but connections were noisy, unstable, and ultimately unsuccessful. Representative SSR recordings from coeloconic sensilla are shown in Fig. 15 below. The simultaneous DC potential tracings obtained along with AC spikes tracings show the depolarizing DC current within the sensillum responsible for generating spikes from the responding OSN (Fig. 15).

4. Discussion

"Our results demonstrate that maxillary and labial palps of both male and female adult *A. glabripennis* have olfactory ability and do not function solely contact chemoreception. The initial impetus for this study stemmed from the results of Graves et al. (2016). They showed *A. glabripennis* males could successfully follow a female-deposited sex-trail

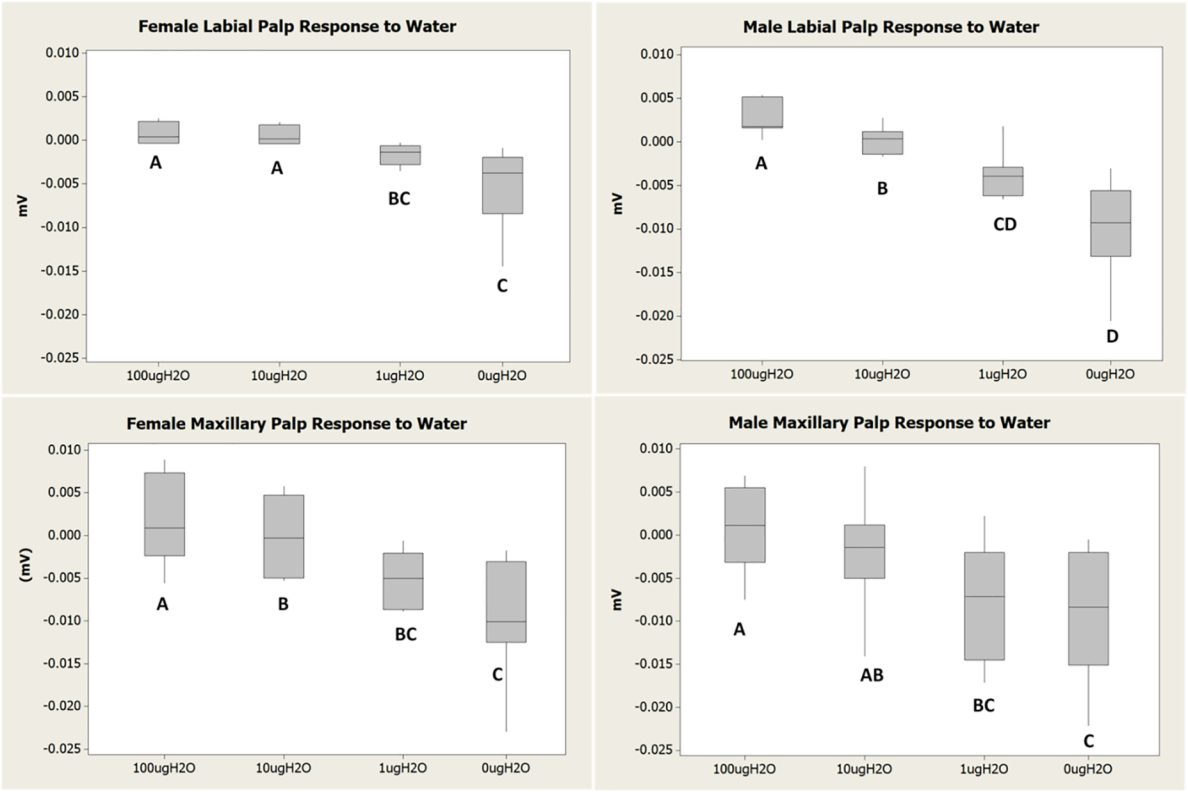


Fig. 9. Box plots of EPGs evoked by puffs from odor cartridges loaded with different dosages of water. Puffs through the cartridges were made using a dry, unhumidified puffing stream, with the pulse exiting the cartridge then entering into the humidified constant air stream flowing over the palps to evoke an EPG. Positive mV values indicate degree of depolarization evoked by the stimulus that moves the OSNs from their normal negative resting potential values in the palps. Negative mV values indicate an increased hyperpolarization of the OSNs (more negative potential) evoked by the stimulus from their already-negative resting potentials. Letters denote statistically different responses, via a GLM followed by a Tukey's test, $p \leq 0.05$, $N = 9$ per palp type per sex.

pheromone only by directly contacting it with their maxillary and labial palps, and not via airborne exposure from as close as a few mm. Because SEM images (Meng, 2014) suggested that sensilla in the apical pit were unlikely to contact surfaces, we predicted olfactory detection of trail pheromones rather than contact chemoreception. Our prediction was not supported by our results, i.e., palps did not respond with significant EPGs to sex-trail pheromone components. However, we learned that the palps responded to many other common general odorants. Furthermore, we suggest that pit flexing may explain how contact chemoreception is enabled by pit sensilla.

Thus, not finding some kind of specific, diagnostic EPG responses to the sex-trail pheromone is consistent with the behavioral findings of Graves et al. (2016) that indicate that contact chemoreception, not olfaction, is what is employed by males to detect and follow the trails of females. It may be that the sensilla in the terminal pits are gustatory, requiring contact with the trail pheromone, and because gustatory tip-recording methods (requiring an entirely different setup and equipment) were not used, we were not able to acquire SSR recordings from the pit-region sensilla.

Evidence that the pit sensilla might be involved with contact chemoreception, possibly including sex-trail-pheromone-following, is related to the discovery of the ability of live ALB to flex the membrane of the apical pits outward from a concave to a convex geometry to now expose the sensilla to direct contact with surfaces. This was a quite a surprise and seems to be quite pertinent to the discussion of how ALB might be detecting the sex-trail pheromone. We observed that ALB can alternatively flex the membrane of the palp tip from an inward concave, to an outward convex, position to allow the sensilla to project out into the environment for potential chemo-sensing. The flexing of the palp-pit membrane strongly implies that it is used for sensing of some kind and leads to a plethora of interesting questions. For instance, the results of Graves et al. (2016) provided evidence that the palps are used to follow the species' sex-trail pheromone by contact alone. Are the sensilla at the palp termini flexed out for trail pheromone contact-sensing? Also, how often does the flexing of the terminal pit membrane happen when the insect is exploring or at rest, rather than in a highly unnatural and stressful situation of being prepared for electrophysiological testing? How does the convex flexing and subsequent exposure of the sensilla relate to contact chemoreception vs. olfaction, especially with regard to detecting and responding to sex-trail pheromone? Are these terminal sensilla primarily gustatory? Our results show that at least some of the chemo-sensing neurons on the palps are capable of olfactory responses, but we were unable to determine whether any sensory neurons in the terminal pit were olfactory. Without electrophysiological evidence one cannot say for certain that the apical pit sensilla are used for chemo-sensing and whether or not that would include olfaction or gustation.

The only direct evidence that OSNs on the palps are capable of olfaction comes from SSR responses from OSNs housed in coeloconic sensilla on the sides of the terminal segment cuticle, not from sensilla clustered in the terminal pits. Therefore, we are only able to conclude at present that the significant EPGs recorded from the palps were due to the summed depolarizations of OSNs on the sides of the palps, and no definite conclusions can be made about the olfactory ability of the apical pits. Coeloconic sensilla are known to house OSNs of many types, including types that are tuned mostly to aldehydes and acids (Benton et al. 2009; Ai et al. 2010; Yao et al. 2005; van Giesen and Garrity, 2017), consistent with the SSR responses found from these sensilla. It may be the coeloconic sensilla are housing the OSNs responding to the more general odorants, but it also seems likely that there are other

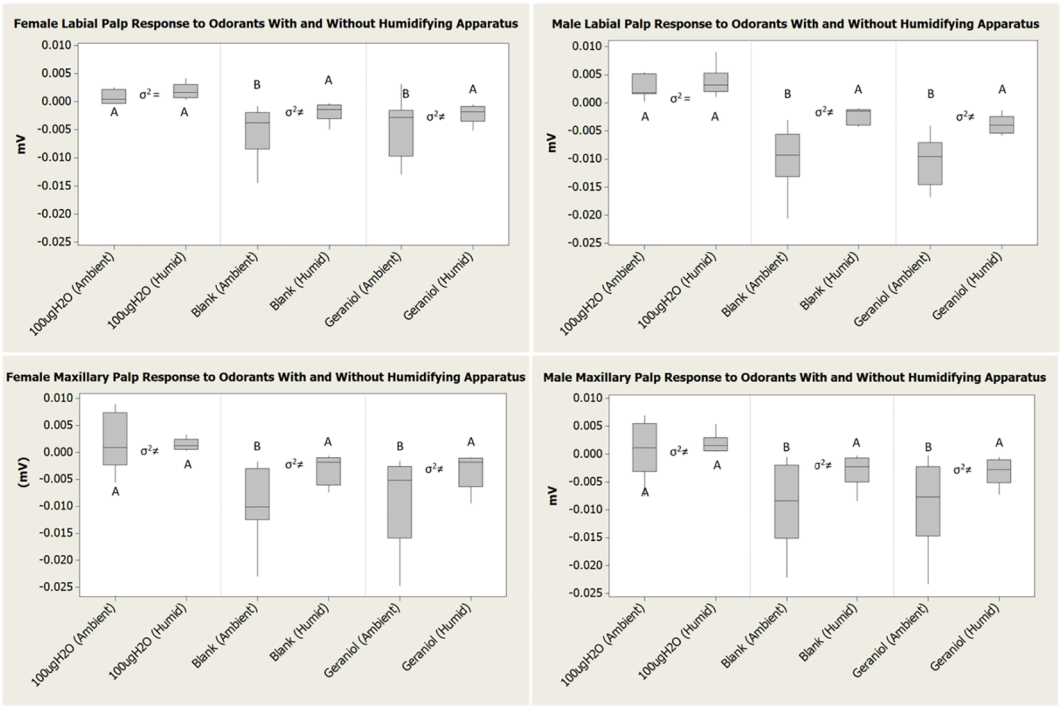


Fig. 10. Effect of decreases or increases in moisture levels imparted by the odor cartridge on the responses, showing EPG responses for blank, geraniol, and water injected with and without a humidified injection stream into a humidified constant airstream. Differing letters denote statistical significance via Wilcoxon signed rank tests performed between the humidified and un-humidified puffs of each odorant, $p \leq 0.05$, $N = 9$. Differences in variances between humidified and un-humidified puffs of each odorant, as measured via an F-test, $p \leq 0.05$, $N = 9$, are also labeled. Geraniol was presented from cartridges loaded at a dosage of 100 µg and water loaded with 100 µl. Increasingly positive mV values indicate an increased degree of depolarization by the stimulus of the normal negative resting potentials of the OSNs in the palps. Increased negative mV values indicate an increased hyperpolarization of the negative resting potentials of the OSNs by the stimulus.

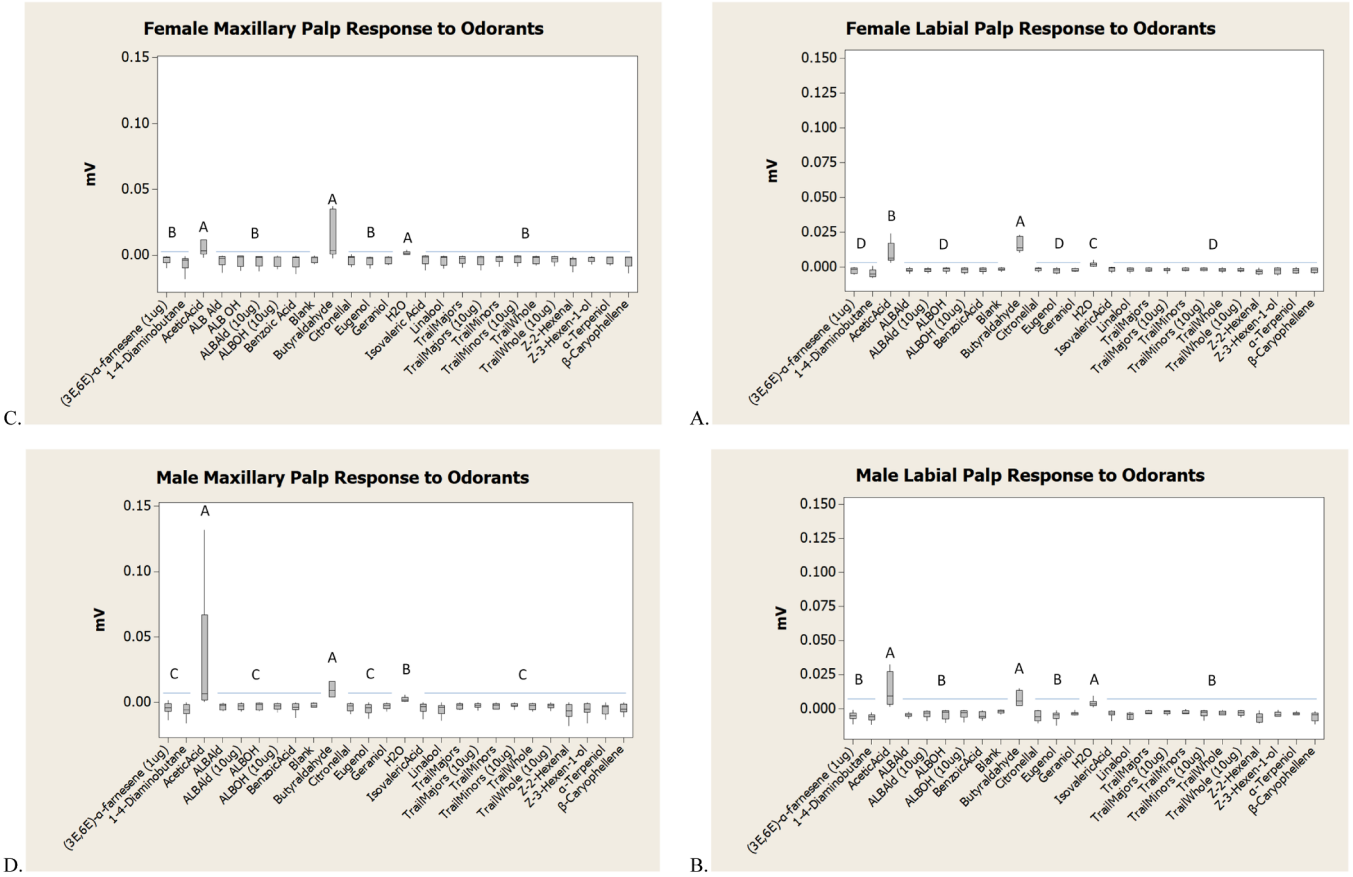


Fig. 11. A-D. EPG responses to different odorants from male and female maxillary and labial palps. EPG amplitudes for different odorants having no letters in common are significantly different via a GLM followed by a Tukey's test, $p \leq 0.05$; $N = 11$. Unless otherwise noted odorants are at 100 µg cartridge loadings and water is loaded at 100 µl.

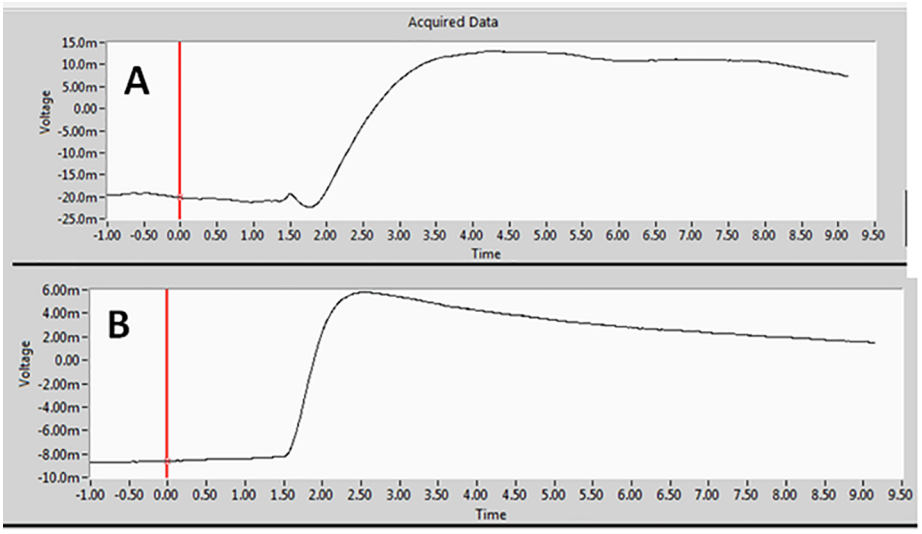


Fig. 12. A) EPG tracing of female maxillary palp response to butyraldehyde showing tonic depolarization. B) EPG tracing of a male maxillary palp response to acetic acid.

types of chemoreceptive sensilla on the palps, other than coeloconic, which will house OSNs responsive to other types of volatiles such as acetates, alcohols, hydrocarbons, etc., and explain the weak but significant EPGs obtained to many different types of odorants tested. Other candidate types of sensilla besides coeloconic have been identified on the palps of adult (Meng, 2014) and larval (Xu et al., 2017) *A. glabripennis* and other cerambycids (Chen et al., 2018) which could be investigated in further studies.

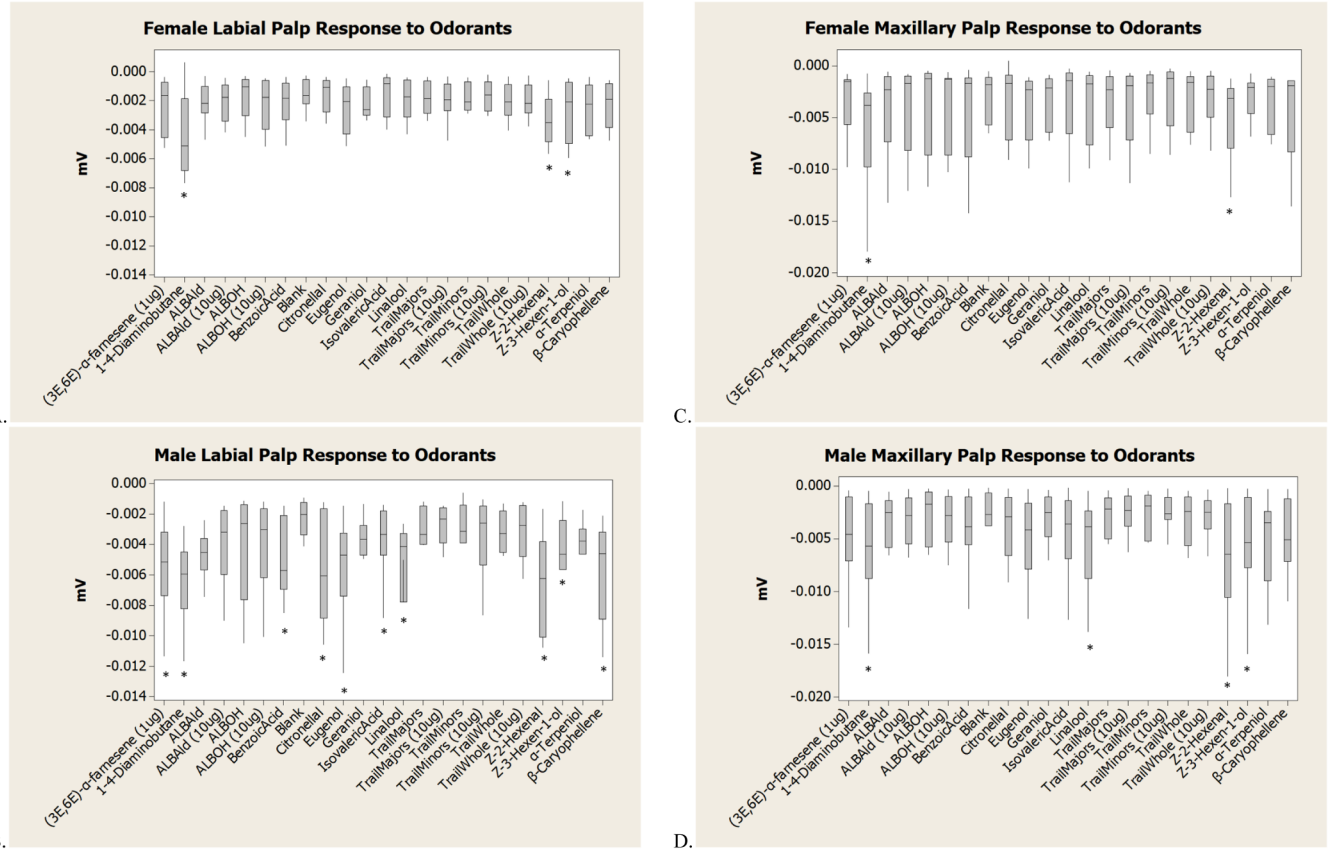
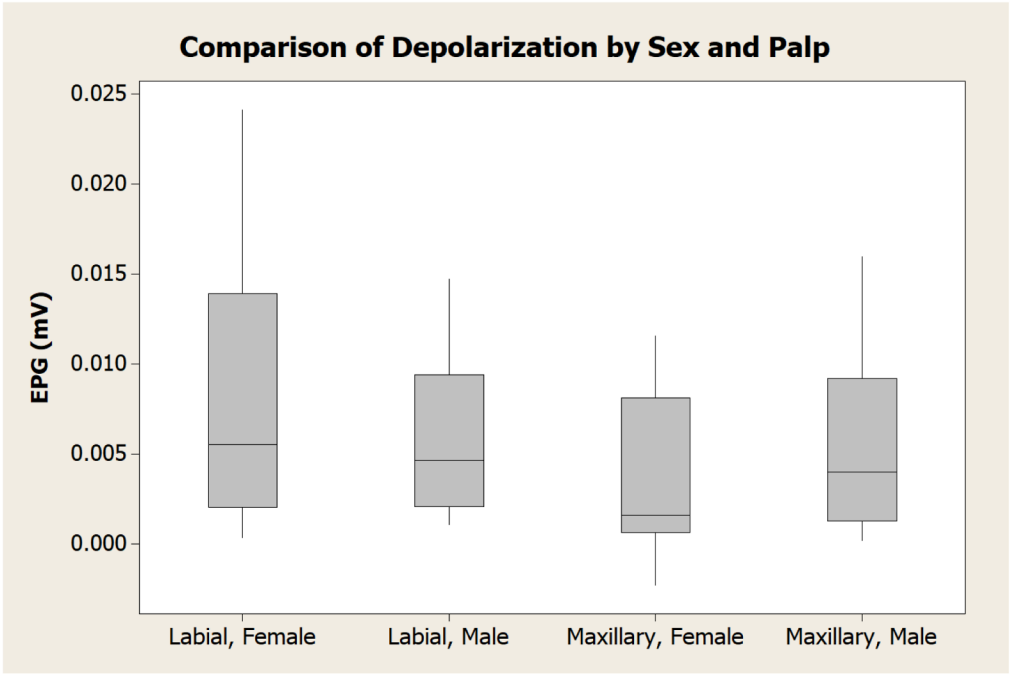


Fig. 13. A-D. EPG responses by male and female maxillary and labial palps to different odorants. "*" indicates that the response was significantly different from the blank response via GLM followed by a Dunnett vs the blank, $p \leq 0.05$; $N = 11$. Unless otherwise noted odorants are at 100 µg strength.

A.



B.

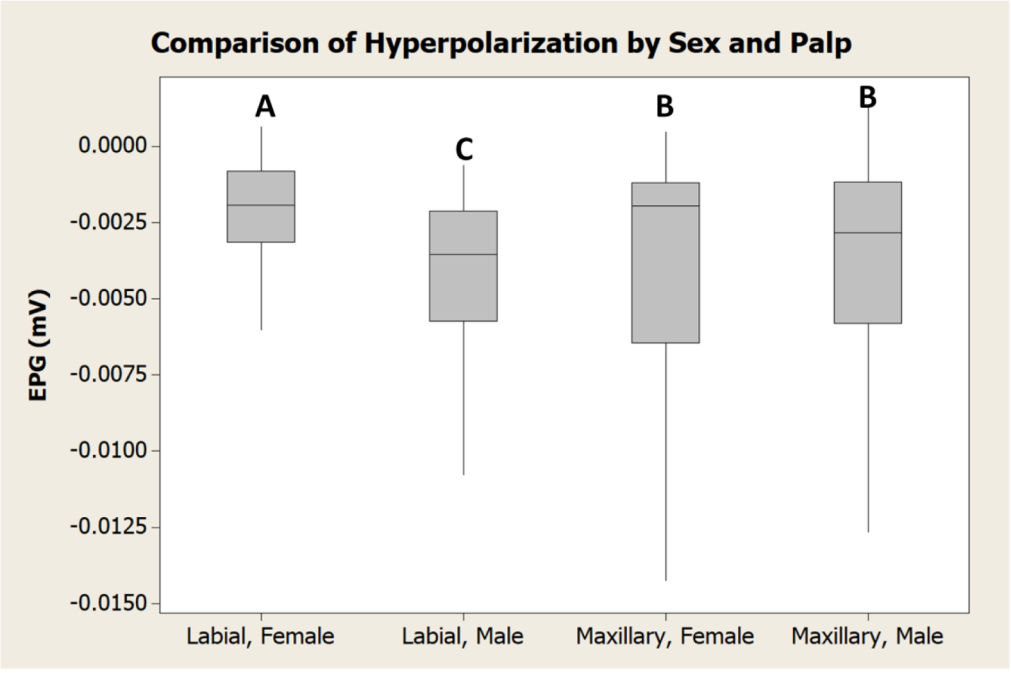


Fig. 14. EPG responses to all odorants tested, from male and female maxillary and labial palps. EPG amplitudes for different odorants having no letters in common are significantly different via a GLM followed by a Tukey's test, $p \leq 0.05$; N = 33 for A) depolarizations (11 individuals × 3 depolarizing odorants) and N = 253 for B) hyperpolarizations (11 individuals × 23 hyperpolarizing odorants).

The labial palps of *A. glabripennis* responded to a greater array of odorants than did the maxillary palps. This differs from the palps of dipterans, especially mosquitoes, whose maxillary palps have olfactory function, but is similar to members of Lepidoptera (Kent et al., 1986), in which labial palps are also used for olfaction. Due to the sample size, our study was not very robust concerning the relatively small panel of odorants we tested, and so it is likely that a wider range of odorant molecules might elicit reactions from the palps due to the broadly tuned characteristic of most olfactory receptors (Hoover et al., 2014) and could be shown more clearly with a larger sample size. Some sexual differences in EPG responses were observed. Male labial palp EPGs were greater in amplitude than those of females, despite the known smaller average size of males (Meng et al., 2015). This result could imply that males have more neurons dedicated to odorant detection in their palps

Table 2
Change in spike frequency (spikes/sec) of OSNs in coeloconic sensilla on maxillary palps of males and females in response to 100ug of odorant. Each row represents a different sensillum.

	Acetic Acid	Blank	butyraldehyde	Water	Trail	
Sensillum A	2		6	2	2	Males
Sensillum B	6	0	9	0	2	
Sensillum C	28	−1	12	0	12	
Sensillum D	3	1	6	−1	5	
mean	*9.75*	*0*	*8.25*	*0.25*	*5.25*	
standard error	*6.14*	*0.50*	*1.44*	*0.63*	*2.36*	
Sensillum A					6	Females
Sensillum B			14			
Sensillum C	0	0	10	20	−3	
Sensillum D	0	−1	2	−4	3	
Sensillum E	2		4	10	12	
Sensillum F	2	0	5	4	0	
Sensillum G	−12	0	−9	8	−6	
Sensillum H	0	2	11	−5	−1	
mean	*−1.33*	*0.2*	*5.29*	*5.5*	*1.57*	
standard error	*2.17*	*0.49*	*2.88*	*3.83*	*2.28*	

than do females. The difference between male and female EPGs might reflect a difference in ability to detect odorants, but it may also simply be an artifact of sample size and thus we are cautious in drawing such a conclusion. A larger study would be necessary to elucidate such a sexual difference more definitively.

The EPG recordings from this study show that palps of *A. glabripennis* are very sensitive to changes in moisture levels, and SSR recordings identified at least one type of sensillum, coeloconic, containing OSNs that respond to water. Also, the multiple short peg sensilla located in the apical pits of the labial and maxillary palps (Fig. 3) might also be hygroreceptors and contribute strongly to the EPGs we recorded in response to water concentrations. According to Altner et al. (1983), many hygroreceptors that have been characterized share the feature of being stubby, non-pore-walled pegs of various lengths that always occur in cuticular depressions of some type. The sensilla in the apical pits seem to fit this classification in all respects except that here we have multitudes of pegs in a single, large pit.

The ability to determine moisture content could be a way for ALB to insure that there is healthy tissue for feeding, oviposition, and subsequent larval feeding as well as to avoid desiccation. The adults of *A. glabripennis* feed on living trees and females lay their eggs in living wood (Meng et al., 2015). Desiccation is a danger for insects and the ability of *A. glabripennis* to identify favorably moist microenvironments might be important (Enjin et al., 2016) especially because it is thought that this species may have initially evolved in moist, riparian habitats (Williams et al., 2004). Access to water is necessary when rearing these insects (David Long, personal communication) and the ability to detect water could also be used to find drinking water sources. It could be argued that response to blanks and water was simply the response of mechanoreceptors to changes in air pressure. However, the dose-dependent response we observed to increasing and decreasing amounts of water puffed over the palps indicates that the EPG responses were elicited by the abundance of water molecules in the puffs. Hygroreceptors have routinely been found on insect antennae through electrophysiological studies (*c.f.,*Pielou, 1940; Altner et al., 1983; Iwasaki et al., 1995; Tichy and Kallina, 2010; Enjin et al. 2016) as well as on palps of larvae and adults (Eilers et al., 2012; Chappuis et al., 2013, respectively). Our findings may inform investigations into hygroreceptive sensory neurons in other species in this family.

For herbivorous beetles like *A. glabripennis*, olfactory detection of acetic acid could play a role in finding suitable feeding/oviposition sites prior to tasting, biting into, or otherwise touching the substrate. Acetic acid is used in this way by other herbivorous insects (George et al., 2016; Ômura & Honda, 2003; Joseph et al., 2009). Acetic acid, along with butylaldehyde and 1,4-diaminobutane, is also associated with decay (Krzymien et al., 1999; Noble et al., 2001; Vass, 2012) and so detection these chemicals with mouthparts could allow *A. glabripennis* to gather information about the state of a food source or oviposition site prior to biting into it. This, along with the detection of other plant-based odorants, could allow these insects to more effectively select higher quality feeding/oviposition sites and avoid less than ideal resources.

Two types of compounds, acids and aldehydes, elicited the strongest EPG responses as well as evoking SSR responses from coeloconic sensilla. The body of knowledge regarding ionotropic receptors (IRs) is growing quickly, and IRs have been identified responding mainly to acids and aldehydes, but also to water, temperature, and gustatory stimuli (van Giesen and Garrity, 2017; Hussain et al., 2016). With such a small number of connections and odorants tested via SSR in our study, we barely scratched the surface of what coeloconic sensilla on the palps are responding to and which sensory neurons are involved. In the EPG and SSR experiments, the finding of tonic depolarization (Fig. 15) and spike initiation by acetic acid and butyraldehyde was a curious thing. Why do these types of molecules activate neural responses so strongly for ten seconds or more but evoke such a short spike train? Perhaps part of the answer lies again in the possibility that OSNs housed in coeloconic sensilla are known to often have IRs expressed on them and not only odorant receptors (ORs) (Benton et al., 2009, Guo et al., 2014, Ai et al., 2010). Perhaps the odorant deactivation mechanisms in the perireceptor environment around IR-employing OSNs within these coeloconic sensilla may not be designed for fast clearing-out of these acid and aldehyde analytes.

The primary conclusion from this study is that the palps in this species, and possibly those of other cerambycid beetles, are likely to be far more involved in sensing volatile compounds, including water molecules, than might have been expected. We are getting only a partial picture of olfaction in *Anoplophora glabripennis*, and likely other cerambycids, when we look only at the antennae, because it appears that the palps also have olfactory capabilities waiting to be better understood.

5. Funding source:

The research performed in this dissertation was funded in part by Cooperative Agreement #16–8130-1430-CA between The Pennsylvania State University and the USDA/APHIS entitled, “Improvement of Traps and Lures for Exotic Wood-boring Beetles.” The funding source provided monies for personnel and equipment and directed the subject matter (wood-boring beetles) upon which those monies were to be used. The funding source did require regular progress reports and accounting of how funding was used. Otherwise it had no involvement in study design, data collection, data analysis, interpretation, or writing of the report.

Declaration of Competing Interest

None.

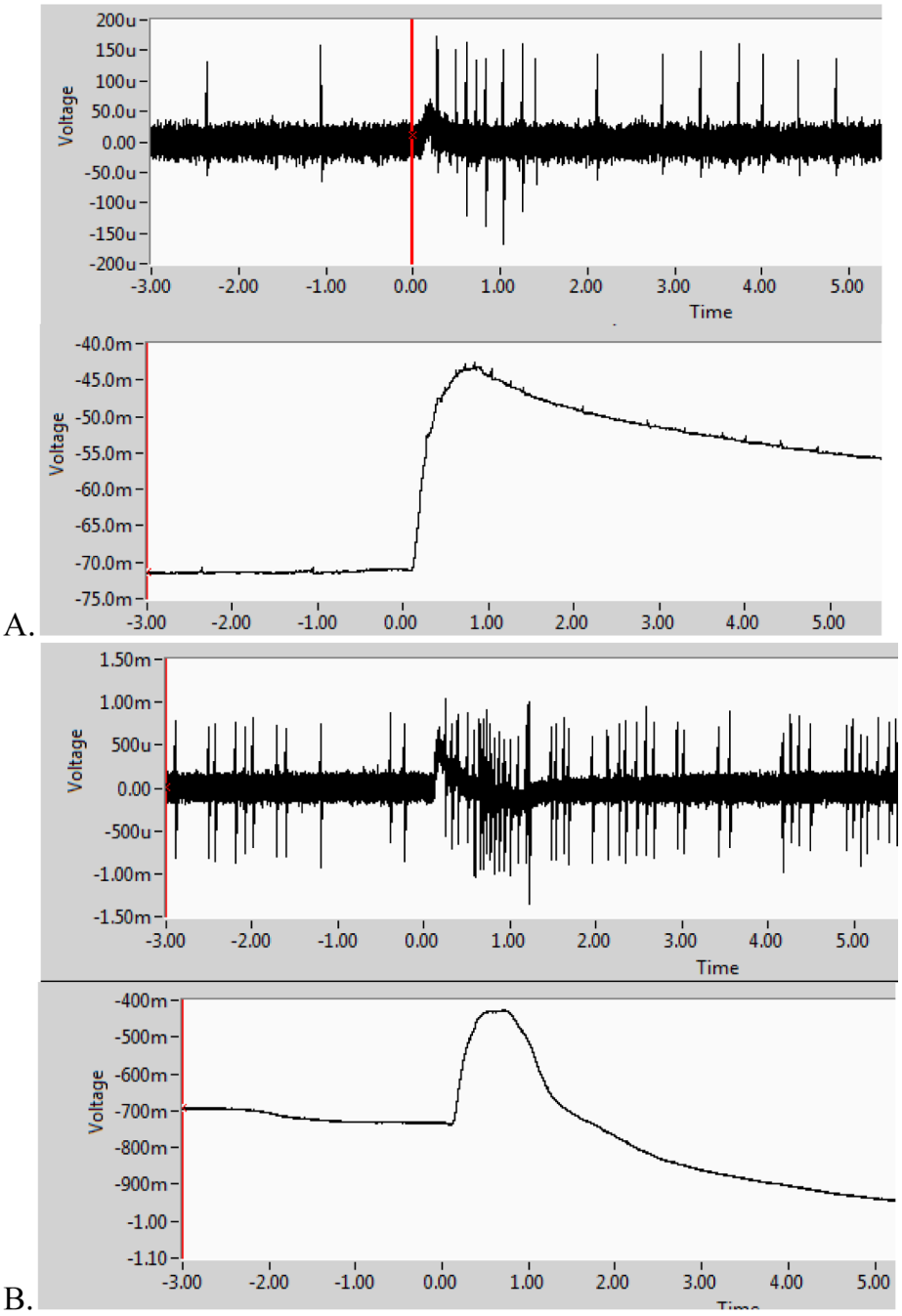


Fig. 15. A. SSR tracing of an ORN from a male coeloconic sensillum responding to butyraldehyde simultaneously showing the DC current from its EPG (or possibly a single-sensillum electrosensillogram (ESG) from this sensillum). B. SSR tracing of an ORN from a female coeloconic sensillum responding to butyraldehyde simultaneously showing the DC current from its EPG (or possibly a single-sensillum electrosensillogram (ESG) from this sensillum).

Acknowledgements

We gratefully acknowledge the funding that supported this study, which came from Cooperative Agreement No. 16-8130-1430-CA between USDA/APHIS and Penn State University entitled “Improvement of Traps and Lures for Exotic Wood-boring Beetles.”

David Long of the Kelli Hoover Laboratory, The Pennsylvania State University, patiently and meticulously reared and maintained the *A.glabripennis* colony for these experiments.

Photographs in Fig. 2 were expertly taken by Carolyn Trietsch of the Andrew Deans Laboratory, The Pennsylvania State University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2019.103905.

References

Ai, M., Min, S., Grosjean, Y., Leblanc, C., Bell, R., Benton, R., Suh, G.S., 2010. Acid sensing by the *Drosophila* olfactory system. Nature 468, 691–695.

Altner, H., Schaller-Selzer, L., Stetter, H., Wohlrab, I., 1983. Poreless sensilla with inflexible sockets. A comparative study of a fundamental type of insect sensilla probably comprising thermo- and hygroreceptors. Cell Tissue Res. 234, 279–307.

Anbutsu, H., Togashi, K., 1997. Oviposition behavior and response to the oviposition scars occupied by eggs in *Monochamus saltuarius* (Coleoptera: Cerambycidae). Appl. Entomol. Zool. 32, 541–549.
Anbutsu, H., Togashi, K., 2000. Deterred oviposition response of *Monochamus alternatus* (Coleoptera: Cerambycidae) to oviposition scars occupied by eggs. Agric. For. Entomol. 2, 217–223.
Ayer, R.K., Carlson, J., 1992. Olfactory physiology in the *Drosophila* antenna and maxillary palp: acj6 distinguishes two classes of odorant pathways. J. Neurobiol. 23, 965–982.
Benton, R., Vannice, K.S., Gomez-Diaz, C., Vosshall, L.B., 2009. Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell 136, 149–162.
Blaney, W.M., 1973. Electrophysiological responses of the terminal sensilla on the maxillary palps of *Locusta migratoria* (L.) to some electrolytes and non-electrolytes. J. Exp. Biol. 60, 275–293.
Chappuis, C.J.F., Béguin, S., Vilmant, M., Guerin, P.M., 2013. Water vapour and heat combine to elicit biting and biting persistence in tsetse. Parasit. Vect. 6, 240.
Chen, J., Zhu, X., Qaio, H., Liu, S., Xu, C., Xu, R., Zhan, W., Li, J., Guo, K., Chen, J., 2018. Ultrastructure of sensilla on the maxillary and labial palps of the adult *Xylotrechus grayii* (Coleoptera: Cerambycidae). Microsc. Res. Tech. 81, 669–680.
Crook, D.J., Lance, D.R., Mastro, V.C., 2014. Identification of a potential third component of the male-produced pheromone of *Anoplophora glabripennis* and its effect on behavior. J. Chem. Ecol. 40, 1241–1250.
de Bruyne, M., Clyne, P.J., Carlson, J.R., 1999. Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. J. Neurosci. 19, 4520–4532.
Dippel, S., Kollmann, M., Oberhofer, G., Montino, A., Knoll, C., Krala, M., Rexer, K.H., Frank, S., Kumpf, R., Schachtner, J., Wimmer, E.A., 2016. Morphological and transcriptomic analysis of a beetle chemosensory system reveals a gnathal olfactory center. Bio. Med. Central Biol. 14, 90.
Eilers, E.J., Talarico, G., Hansson, B.S., Hilker, M., Reinecke, A., 2012. Sensing the underground – ultrastructure and function of sensory organs in root-feeding *Melolontha melolontha* larvae. PLoS ONE 7 e41357.
Enjin, A., Zaharieva, E.E., Frank, D.D., Mansourian, S., Suh, G.S.B., Gallio, M., Stensmyr, M.C., 2016. Humidity sensing in *Drosophila*. Curr. Biol. 26, 1352–1358.
Fan, J., Kang, L., Sun, J., 2007. Role of host volatiles in mate location by the Japanese pine sawyer, *Monochamus alternatus* hope (Coleoptera: Cerambycidae). Environ. Entomol. 36, 58–63.
George, J., Robbins, P.S., Alessandro, R.T., Stelinski, L.L., Lapointe, S.L., 2016. Formic and acetic acids in degradation products of plant volatiles elicit olfactory and behavioral responses from an insect vector. Chem. Senses 41, 325–338.
Giglio, A., Perrotta, E., Talarico, F., Zetto Brandmayr, T., Ferrero, E.A., 2013. Sensilla on maxillary and labial palps in a helicophagous ground beetle larva (Coleoptera, Carabidae). Acta Zool. (Stockholm) 94, 324–330.
Graves, F., Baker, T.C., Zhang, A., Keena, M., Hoover, K., 2016. Sensory aspects of trail-following behaviors in the Asian longhorned beetle, *Anoplophora glabripennis*. J. Insect Behav. 29, 615–628.
Guo, M., Krieger, J., Große-Wilde, E., Mißbach, C., Zhang, L., Breer, H., 2014. Variant ionotropic receptors are expressed in olfactory sensory neurons of coeloconic sensilla on the antenna of the desert locust (Schistocerca gregaria). Int. J. Biol. Sci. 10, 1–14.
Hall, D.R., Cork, A., Phythian, S.J., Chittamuru, S., Jayarama, B.K., Venkatesha, M.G., Sreedharan, K., Vinod Kumar, P.K., Seetharama, H.G., Naidu, R., 2006. Identification of components of male-produced pheromone of coffee white stemborer, *Xylotrechus quadripes*. J. Chem. Ecol. 32, 195–219.
Hoover, K., Keena, M., Nehme, M., Wang, S., Meng, P., Zhang, A., 2014. Sex-specific trail pheromone mediates complex mate finding behavior in *Anoplophora glabripennis*. J. Chem. Ecol. 40, 169–180.
Hussain, A., Zhang, M., Üçpunar, H.K., Svensson, T., Quillery, E., Gompel, N., Ignell, R., Grunwald-Kadow, I.C., 2016. Ionotropic chemosensory receptors mediate the taste and smell of polyamines. PLoS Biol. 14 e1002454.
Iwasaki, M., Itoh, T., Yokohari, F., Tominaga, Y., 1995. Identification of antennal hygroreceptive sensillum and other sensilla of the firefly, *Luciola cuciata*. Zool. Sci. 12, 725–732.
Joseph, R.M., Devineni, A.V., King, I.F.G., Heberlein, U., 2009. Oviposition preference for and positional avoidance of acetic acid provide a model for competing behavioral drives in *Drosophila*. Proceed. Natl. Acad. Sci. U.S.A. 106, 11352–11357.
Keena, M.A., 2005. Pourable artificial diet for rearing *Anoplophora glabripennis* (Coleoptera: Crambycidae) and methods to optimize larval survival and synchronize development. Ann. Entomol. Soc. Am. 98, 536–547.
Kent, K.S., Harrow, I.D., Quartararo, P., Hildebrand, J.G., 1986. An accessory olfactory pathway in Lepidoptera: the labial pit organ and its central projections in *Manduca sexta* and certain other sphinx moths and silk moths. Cell Tissue Res. 245, 237–245.
Krzymien, M., Day, M., Shaw, K., Zaremba, L., 1999. An investigation of odors and volatile organic compounds released during composting. J. Air Waste Manag. Assoc. 49, 804–813.
Li, D., Tokoro, M., Nacashima, T., 1999. Mechanism of adult action and mating in *Anoplophora glabripennis* (Motsch.). J. Beijing Forest. University 21, 33–36.
Liendo, C., Morillo, F., Sánchez, P., Muñoz, W., Guerra, J., Cabrera, A., Hernández, J.V., 2005. Olfactory behavior and electroantennographic responses of the cocoa beetle, *Steirastoma breve* (Coleoptera : Cerambycidae). Flor. Entomol. 88 (2), 117–122.
Meng, P.S., Hoover, K., Keena, M.A., 2015. Asian longhorned beetle (Coleoptera: Cerambycidae), an introduced pest of maple and other hardwood trees in North America and Europe. J. Integrat. Pest Manage. 6, 4.
Meng, P.S., 2014. Where's that smell? Trapping and sensory biology of the Asian longhorned beetle, *Anoplophora glabripennis*. Master's thesis. State University, University Park, PA, Pennsylvania.
Noble, R., Hobbs, P.J., Dobrovin-Pennington, A., Misselbrook, T.H., Mead, A., 2001. Olfactory response to mushroom composting emissions as a function of chemical concentration. J. Environ. Qual. 30, 760–767.
Ômura, H., Honda, K., 2003. Feeding responses of adult butterflies, *Nymphalis xanthomelas*, Kaniska *canace* and *Vanessa indica*, to components in tree sap and rotting fruits: synergistic effects of ethanol and acetic acid on sugar responsiveness. J. Insect Physiol. 49, 1031–1038.
Pielou, D.P., 1940. The humidity behaviour of the mealworm beetle, *Tenebrio molitor* L. II. The humidity receptors. J. Exp. Biol. 17, 295–306.
Syed, Z., Leal, W.S., 2007. Maxillary palps are broad spectrum odorant detectors in Culex quinquefasciatus. Chem. Sens. 32, 727–738.
Talarico, F., Pg, Giulianini, Brandmayr, P., Giglio, A., Masala, C., Sollai, G., Zetto Brandmayr, T., Solari, P., 2010. Electrophysiological and behavioural analyses on prey searching in the myrmecophagous carabid beetle *Siagona europaea* (Dejean 1826). Ethol. Ecol. Evolut. 22, 375–384.
Tichy, H., Kallina, W., 2010. Insect hygroreceptor responses to continuous changes in humidity and air pressure. J. Neurophysiol. 103, 3274–3286.
Toshova, T.B., Subchev, M., Abaev, V., Vuts, J., Imrei, Z., Koczor, S., Galli, Z., Van De Ven, R., Tóth, M., 2016. Responses of *Pseudovadonia livida* adults to olfactory and visual cues. Bull. Insectol. 69, 161–172.
van Giesen L, Garrity PA. 2017. More than meets the IR: the expanding roles of variant Ionotropic Glutamate Receptors in sensing odor, taste, temperature and moisture. F1000Research 6, 1753.
Vass, A.A., 2012. Odor Mortis. Forensic Sci. Int. 222, 234–241.
Wei, J., Zhou, Q., Hall, L., Myrick, A., Hoover, K., Shields, K., Baker, T.C., 2018. Olfactory sensory neurons of the Asian longhorned beetle, *Anoplophora glabripennis*, specifically responsive to its two aggregation-sex pheromone components. J. Chem. Ecol. 44, 637–649.
Williams, D.W., Lee, H.P., Kim, I.K., 2004. Distribution and Abundance of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in Natural *Acer* Stands in South Korea. Environ. Entomol. 33, 540–545.
Xu, L., Zhang, L., Yang, Y., Ren, L., Wang, T., Zong, S., 2017. Morphology of antennal, maxillary palp and labial palp sensilla in different larval instars of the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae). Acta Zool. 98, 20–31.
Yao, C.A., Ignell, R., Carlson, J.R., 2005. Chemosensory coding by neurons in the coeloconic sensilla of the *Drosophila* antenna. J. Neurosci. 25, 8359–8367.