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Research article

Response of *Pediculus humanus capitis* (Phthiraptera: Pediculidae) to Volatiles of Whole and Individual Components of the Human Scalp

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Abstract

The head louse *Pediculus humanus capitis* (De Geer) (Phthiraptera: Pediculidae) is a cosmopolitan human ectoparasite causing pediculosis, one of the most common arthropod parasitic conditions of humans. The mechanisms and/or chemicals involved in host environment recognition by head lice are still unknown. In this study, we evaluated the response of head lice to volatiles that emanate from the human scalp. In addition, we identified the volatile components of the odor and evaluated the attractive or repellent activity of their pure main components. The volatiles were collected by means of Solid Phase microextraction and the extract obtained was chemically analyzed by gas chromatograph-mass spectrometer. Twenty-four volatile were identified in the human scalp odor, with the main compounds being the following: nonanal, sulcatone, geranylacetone, and palmitic acid. Head lice were highly attracted by the blend human scalp volatiles, as well as by the individual major components. A significant finding of our study was to demonstrate that nonanal activity depends on the mass of the compound as it is repellent at high concentrations and an attractant at low concentrations. The results of this study indicate that head lice may use chemical signals in addition to other mechanisms to remain on the host.

Keywords: *Pediculus humanus capitis*, human scalp volatiles, attractant activity, repellent activity

The human head louse *Pediculus humanus capitis* (De Geer) (Phthiraptera: Pediculidae) is a cosmopolitan human ectoparasite that causes pediculosis, one of the most prevalent parasitic diseases of humans. Head louse infestation is annoying and may cause itching, loss of sleep, and social sanctioning (Burgess 2004). Pediculosis incidence has increased worldwide as a result of control product failures owing to insecticide resistance, improper application, formulation changes, and misdiagnoses (Burgess 2009). Head lice mainly affect children 5- to 14-yr old but also affect children outside the age range, as well as adults (Toloza et al. 2009). Transmission of head lice occurs by direct host-to-host contact and by fomites (Speare et al. 2002).

Blood-feeding insects use human skin secretions and metabolites from interaction with skin microflora as cues. These blends contain up to 400 volatile chemicals including alkanes, alkenes, alcohols, aldehydes, ketones, acids, and 3′-lactones (Nicolaides 1974, Goetz et al. 1987, Penn et al. 2007, Gallagher et al. 2008). Among these compounds, 1-octen-3-ol, l-lactic acid, and C3–C5 carboxylic acids are known to elicit attraction of hematophagous insects such as mosquitoes, biting midges, kissing bugs, and tsetse flies (Lehane 2005).

Regarding human lice, Wigglesworth (1941) observed that body lice [*Pediculus humanus humanus*, Linneus, 1758 (Phthiraptera: Pediculidae)] had a preference for human body odor when they were exposed to different odors in an experimental area. Moreover, Mumcuoglu et al. (1986) found that body lice had a tendency to aggregate when exposed to louse excrements and identified the active attractant agents as hemoglobin, xanthine, hypoxanthine, uric acid, and ammonium salts.

Head lice are specific obligate ectoparasites that initially contact a host as a result of physical contact between two heads or by fomites. According to Takano-Lee et al. (2005), adults, nymphs can move to a new host in situations such as host grooming, during which lice exhibit a 'flee response' or a rapid movement away from the disturbance area. Thus, head lice do not need to perform an active long-distance host search like most hematophagous insects. However, Ortega-Insaurralde et al. (2016) reported that the exposure of head lice to filter papers impregnated with human odor induced them to decrease their average locomotor activity and to remain arrested on the treated paper. No difference in the response of different sex or age of the host was observed.

The main goal of the present work was to evaluate the behavioral response of head lice to the volatiles of the whole human scalp odor and to the major volatile components.

Materials and Methods

Insects

Head lice were collected (~400 lice in total) from the heads of infested children from elementary schools (Argentinean Government owned and non-fee-paying) in Buenos Aires, using a fine toothed antilouse comb. Only pupils whose parents had given informed consent for participation were examined; the decision to participate (or not to participate) in the research was clearly established for each pupil. For those students willing to participate, the entire head was examined carefully with special attention paid to the nape of the head and behind the ears. The protocol for lice collection was approved by the ad hoc committee of the Centro de Investigaciones de Plagas e Insecticidas (UNIDEF, Buenos Aires, Argentina) and archived in our institution (# BA20061995ARG, June 1995; Picollo et al. 1998). The collected lice were transported to the laboratory for selection. Insects that had damage to the legs or antennae were discarded for the tests. After this analysis, a subset of 258 (between adult males and females) was selected. During the selection process, the selected insects were kept in plastic trays in an environmental chamber with controlled relative humidity (60–70%) and temperature (17 ± 1°C). These conditions were reported as optimal for the survival of insects in the laboratory (Picollo et al. 1998).

Collection of Human Scalp Volatile Chemicals

Human scalp secretions, used for both behavioral bioassays and the chemical analyses, were collected from an adult-young male volunteer who had washed his hair with neutral pH shampoo (Biferdil, Argentina) 48 h previous to the study. For behavioral tests, a filter paper square Whatman N 1 (1.5 × 1.5 cm) (Buckinghamshire, United Kingdom) was gently rubbed against the scalp surface of the same volunteer for 30 s and placed in one arm of the olfactometer. A similar but untreated filter paper was placed in the other arm of the olfactometer (blank without odor). For the chemical analysis, volatiles were collected by fixing an oven bag previously warmed at 180°C (30 × 45 cm; B.P Premium, Argentina) to the upper part of the head for an hour. During the last 10 min, the volunteer performed moderate physical activity to stimulate perspiration. Volatiles were collected (in duplicate) by means of a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30 µm) solid phase micro-extraction (SPME) fiber (Supelco) punctured through the bag in order to expose its active phase to the headspace for 15 min in a temperature-controlled environment (30°C).

Chemical Analysis

The collected volatiles were analyzed on a Shimadzu QP2010-Ultra-Gas chromatograph-Mass spectrometer (GC-MS). The GC was equipped with a polar DB-WAX capillary column (30 m, 0.32 mm i.d, d.f 0.25 µm, Agilent Technologies; Santa Clara, CA) with helium as a carrier (total flow 4.82 ml/min). The fiber was injected into the GC split less injector kept at 240°C. The GC oven temperature was programmed from 50°C (4-min hold), followed by a ramp of 8°C/min to 230°C, and held isothermal for 5 min. The detector was operated at 70 eV, scanning from 40 m/z to 350 m/z, with 245°C interface temperature. The chemical characterization of single volatile compounds was carried out using standard reference samples obtained from Sigma–Aldrich (St Louis, MO), by comparing its retention index (RI) with literature data and/or by the comparison and analysis of the mass spectrum (MS) against the Wiley Mass Spectra Library (McLafferty 2005).

Compounds found in each total ion chromatogram were separately normalized as follows: the MS of all components arising from siloxanes, room air, liquid soap, solvents, (e.g., traces of chloroform, column, and septa), as well as solvents commonly employed in cosmetic products and room air products (e.g. 2- butoxy ethanol) were not considered in the analysis. All the areas of the compounds that were not eliminated were added and then divided by each individual area to know their proportion. The compounds identified with the largest relative areas were selected to be evaluated individually.

Behavioral Bioassay

The evaluation of the behavioral response of the collected head lice to volatiles from human scalp was performed in an adapted T-tube olfactometer. The olfactometer was set in a closed chamber with low-intensity light (21 lux) and controlled temperature (30°C) (Ortega-Insaurralde et al. 2015). The insect behavior was recorded with a weatherproof infrared camera (KIR-J639CE20, Sony, China) connected to a monitor (LG, China) and a digital video recorder (DVR5104HE, Dajua Technology Co. Ltd, Hangzhou, China). A filter paper square Whatman N 1 (1.5 × 1.5 cm) previously rubbed over the volunteer's scalp for a period of 30 s was placed into a 2-ml vial connected to one arm of the olfactometer, and an untreated control paper square was placed into the vial connected to the other arm of the olfactometer (Fig. 1). The sample was randomly placed in the right and the left arm of the olfactometer.

The airstream through the olfactometer was 5 cm/s as measured with an anemometer. Air was filtered through activated charcoal and humidified through a washer flask. Before starting an experimental session, the olfactometer was thoroughly cleaned with pure ethanol. After each test, the vials were cleaned with pure ethanol and after four trials the entire device was cleaned.

Each individual adult head louse was placed in the center of the olfactometer through the central opening and allowed to freely crawl for 3.5 min. At that point, the location of the louse in relation to the two side arms of the olfactometer was recorded by assigning a binary value (1 = proximity to the impregnated paper or 0 = proximity to the untreated paper). The test evaluated the movement of the louse to the clean paper or to the paper impregnated with human odor (n = 33). After this phase of the experiment, the insects used were discarded. In order to exclude a possible bias toward one side of the olfactometer, a control experiment was also performed by placing one clean filter paper into each arm (n = 33).

To evaluate the behavior response of head lice to the main components of human scalp individually, solutions from pure chemicals were prepared in acetone (99.8%, Merck Darmstadt, Germany) at 0.001, 0.01, 0.1, and 1 mg/ml. Whatman filter papers were impregnated with 10 µl of each dilution to obtain final masses of 0.01, 0.1, 1, and 10 µg component on the filter paper. Each concentration was evaluated versus filter papers impregnated with acetone. In total, 12 replicates were performed on each mass of the identified components of the human scalp odor (n = 192 lice in total). Dose–response curves were calculated to assess the effect of the different masses on the probability of choice (proximity to the source). Finally, in compounds detected in significant concentration, differences at behavioral response between treatment at the highest dose evaluated (10 µg) and at the control (0 µg) were analyzed (12 replicates of each).

Data Analysis

All binary data (proximity to the source or proximity to the clean paper) from the olfactometer were analyzed using generalized linear model (GLM) with binomial error structure with n = 1 (Bernoulli distribution) and logit link function (Zuur et al. 2009). All analyses were conducted using lme4: linear mixed effects (Bates et al. 2013) and MASS packages (Venables and Ripley 2002) from the R software (R Core Team 2013).

Assumptions of independent observations and randomness in the model were assured by experimental design (each louse was assigned only to one trial and the treated paper was assigned randomly to the right or left side of the olfactometer). Absence of outliers was assessed from Pearson residuals versus prediction graphics and absence of influential data was checked in Cook's graphic, where distance values were always ≤1.

Results

Chemical Analyses of Human Scalp

The volatile compounds of the human head scalp identified by GC-MS are shown in Table 1. The analyses revealed high proportions (relative areas ≥ 10%) of nonanal, 6-methyl 5-heptene-2-one (sulcatone),

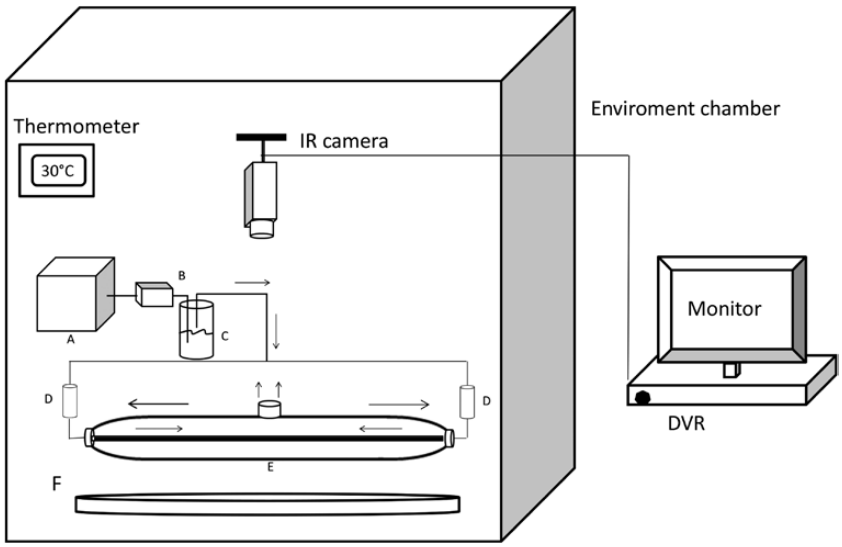

Fig. 1. Dual choice 'T'-shaped olfactometer used to test behavioural effect of adult *P. humanus capitis*. (A) air pump, (B) charcoal filter, (C) humidifier, (D) 2-ml hermetic glass vials, (E) decision point, and (F) hot plate. Arrows indicate the air flow directions.

Table 1. Volatile compounds detected from human scalp by GC-MS analysis

#	Relative RI	Name	Proportions	Literature RI	STD	Match (%)	References
1	1275	Octanal	**	1277	a	97	Khan and Verma (2009)
2	1306	Sulcatone	***	1318	a	98	Yamaguchi and Shibamoto (1981)
3	1356	Nonanal	***	1350	a	97	Yamaguchi and Shibamoto (1979)
4	1435	Acetic acid	**	1433	a	97	Gancel et al. (2005)
5	1450	Myrcenol	**	1455	a	97	Babushok et al. (2011)
6	1455	Decanal	**	1448	a	97	Yamaguchi and Shibamoto (1979)
7	1489	Propanoic acid	**	1486	a	98	Shibamoto et al. (1981)
8	1582	Isobutyric acid	*	1587	a	97	Viña and Murillo (2003)
9	1620	Butanoic acid, 3-methyl	**	1623	a	93	Shibamoto et al. (1981)
10	1803	Hexanoic acid	**	1836	a	94	Babushok et al. (2011)
11	1814	Geranylacetone	***	1816	a	97	Yamaguchi and Shibamoto (1981)
12	1906	Hexanoic acid, 2-ethyl	**	1910	a	96	Babushok et al. (2011)
13	1976	1-Dodecanol	**	1967	a	94	Babushok et al. (2011)
14	2042	Octanoic acid	**	2050	a	96	Rezende and Fraga (2003)
15	2142	Nonanoic acid	**	2156	a	96	Tatsuka et al. (1990)
16	2145	1-Tetradecanol	**	2140	a	97	Hanai and Hong (1989)
17	2269	Decanoic acid	**	2273	a	97	Babushok et al. (2011)
18	2347	1-Hexadecanol	**	2342	a	97	Babushok et al. (2011)
19	2482	Dodecanoic acid	**	2486	a	97	Babushok et al. (2011)
20	2578	1-Octadecanol	**	2586	a	96	Babushok et al. (2011)
21	2662	Tetradecanoic acid	**	2686	a	97	Babushok et al. (2011)
22	2820	Pentadecanoic acid	**	2822	a	96	Babushok et al. (2011)
23	2901	Palmitic acid	***	2910	a	97	Babushok et al. (2011)
24	2919	6-Octadecenoic acid	*	-	b	97	

Proportions based on relative areas.
'a' ID confirmed also by comparison with synthetic standard.
'b' synthetic standard.
***Greater than 10%.
**1–10%.
*Less than 1%.

hexadecanoic acid (palmitic acid), and (*E-E*)-5,9-undecadien-2-one, 6,10-dimethyl (geranylacetone) (Fig. 2). Compounds detected in minor proportions (relative areas between 1 and 10%) were octanal, decanal, 7-octen -2-ol-2,6-dimethyl (myrcenol), propanoic acid, butanoic acid 3-methyl (isovaleric acid), 2-ethyl hexanoic acid, octanoic acid, nonanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, 1-dodecanol, 1-tetradecanol, 1-hexadecanol, and 1-octadecanol. Two compounds were found in proportions ≤ 1%: isobutyric acid and an unsaturated 18 C acid (presumably 6-octadecenoic acid), although MS did not define the position of the double bond.

Behavioral Assay

The response of head lice to the volatiles released by filter papers treated with the whole human odor or its main components was tested by a T-tube olfactometer. Provided with the option to move toward the untreated paper or the human odor, the head lice showed significant response toward the human odor air (P = 0.002). The model probability of choice (treatment) showed significant difference compared to the null model (P = 0.001415).

The observed dose–response curves for the most abundant compounds identified in volatile emissions are shown in Fig. 3 (48 lice were used for each compound). The evaluation of the effect of the mass (0.001, 0.1, 1, and 10 µg) on the probability of choice indicated that for sulcatone, nonanal, and geranylacetone the attraction decreased as the mass increased (negative slopes in Equations 1–3; Fig. 3A–C). In contrast, for palmitic acid the trend is the inverse (positive slope in Equation 4; Fig. 3D). However, the only model for nonanal was significant on the probability of choice (P = 0.00622). For this compound, higher concentrations produced repellency and lower yielded attraction. The model probability of choice-mass of nonanal differed significantly from null model (P = 0.001687). Additionally, nonanal showed significant difference on the probability of choice between the control and the highest dose analyzed (P = 0.0406).

$$\text{Sulcatone}: \quad \text{Probability of choice} = \frac{e^{0.51240-0.12501*\text{mass}}}{1+e^{0.51240-0.12501*\text{mass}}} \tag{1}$$

$$\text{Nonanal}: \quad \text{Probability of choice} = \frac{e^{0.79028-0.24260*\text{mass}}}{1+e^{0.79028-0.24260*\text{mass}}} \tag{2}$$

$$\text{Geranylacetone}: \quad \text{Probability of choice} = \frac{e^{0.15706-0.08934*\text{mass}}}{1+e^{0.15706-0.08934*\text{mass}}} \tag{3}$$

$$\text{Palmitic acid}: \quad \text{Probability of choice} = \frac{e^{-0.25174+0.06068*\text{mass}}}{1+e^{-0.25174+0.06068*\text{mass}}} \tag{4}$$

Discussion

This work represents the first evidence of head louse response to volatiles as the insects showed a clear preference to the arm of the olfactometer containing human odor compared to the neutral odors arm.

Although human lice are permanent parasites that spend all stages of their life cycle on the host, the simple continuation of pediculosis is evidence that there is dispersion of parasites between different hosts. Not only can head lice accidentally fall from the host, but there is evidence that human lice may leave a host when there is severe temperature changes, indicating that their host is unhealthy and affecting the development of the lice colony (Buxton 1946). Also, lice, can disperse to a new host in situations such as host grooming, when they actively crawl away from the disturbance area (Takano-Lee et al. 2005). In these noninfrequent situations, chemical cues may acquire a potentially primary role. Additionally, body lice can quest and move to a new host by using heat as a locating signal (Wigglesworth 1941). Although not closely related to *Pediculus*, dove body chewing lice [Phthiraptera: Mallophaga: *Columbicola columbae* (Linneo 1758)] was reported by Johnson et al. (2011) to have accidental dislodgement from the host. Dove body lice complete the entire life cycle on the host but may accidentally be dislodged from the bodies of preening pigeons, fall to the ground and climb onto a new host, effectively resulting in a dispersion between hosts. These background results together with the ones presented here suggest that human lice use heat and host odor to identify a human host during accidental displacement.

The response of head lice to nonvolatiles plus volatile components of human scalp was recently reported by Ortega-Insaurralde et al. (2016), who established the attraction to filter paper with human odor. The authors found that the whole scalp components (high and low volatilities) induced head lice to decrease average locomotor activity and remain arrested on the treated paper, probably due to the association between human odor and food source. Unfortunately, this study did not analyze the response toward isolated components of the human scalp odor. The results of the present study in addition to those of Ortega-Insaurralde et al. (2016) suggest that head lice may be attracted by volatiles of human scalp and are subsequently arrested by less volatile components.

GC-MS was used to identify volunteer volatile compounds for further evaluation. Major compounds found were sulcatone,

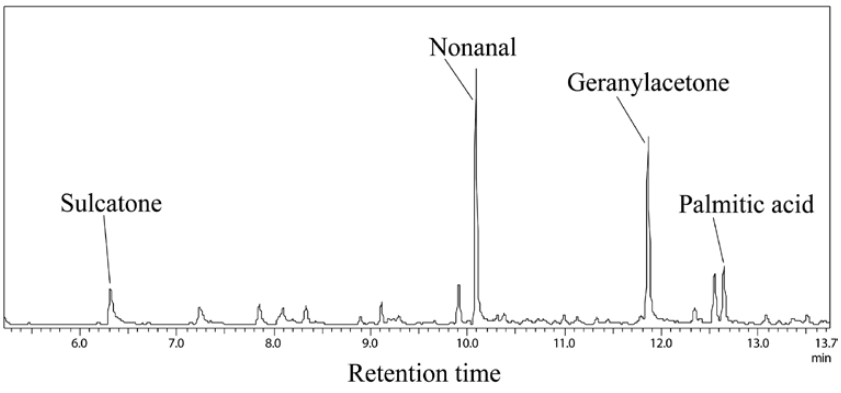

Fig. 2. GC-MS trace of volatiles collected from a volunteer scalp by SPME. Sulcatone, nonanal, geranylacetone, and palmitic acid were the major volatile compounds.

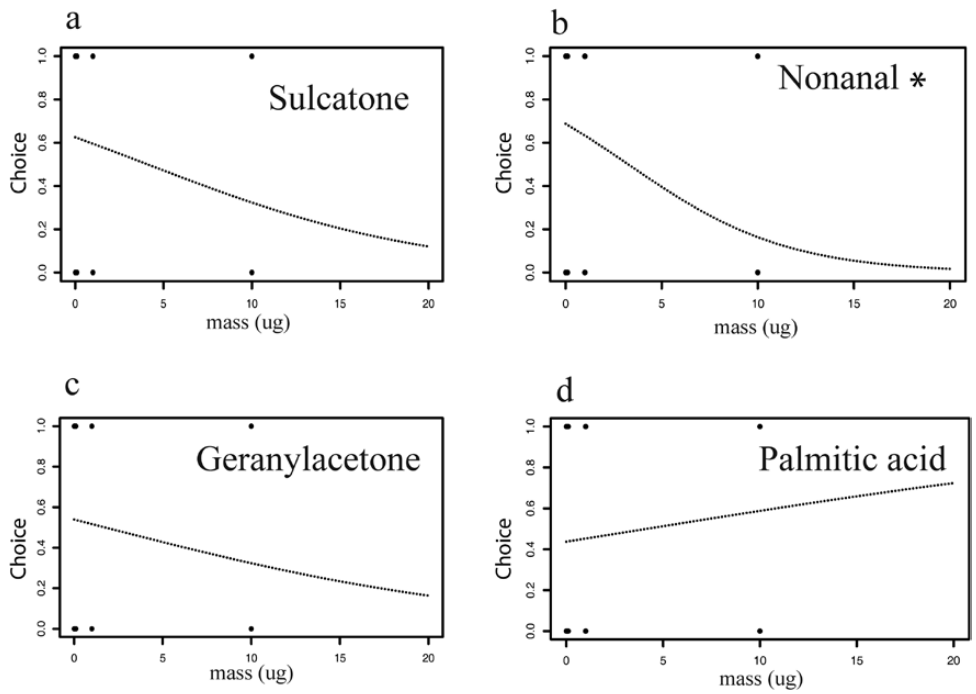

Fig. 3. Dose–response curves of principal compounds. The logistic model evaluates the probability of choice (proximity to the source) as a function of mass of the four compounds: (A) sulcatone, (B) nonanal, (C) geranylacetone, and (D) palmitic acid. Although sulcatone, nonanal, and geranylacetone display the same trend, only nonanal showed significant differences between masses.

nonanal, geranylacetone, and palmitic acid. Cautions were applied to eliminate contamination of the extracts by controlling the washing regimes and avoiding the use of industrial or cosmetic chemicals by the subject participating in the trials. Although they may have different sources, it is well known that sulcatone, nonanal, and geranylacetone are present in human odor (Penn et al. 2007, Gallagher et al. 2008).

An important finding was that nonanal activity significantly depends on its mass, showing repellency at high concentrations and attractiveness at low concentrations. This compound was previously described as a host odor constituent and is detected by other arthropods such as the kissing bug *Triatoma infestans* (Stal) (Hemiptera: Reduviidae) (Guerenstein and Guerin 2001) and the biting midge *Culicoides impunctatus* (Goetghebuer) (Logan et al. 2009) . The repellent activity of another aldehyde (piperonal) on head lice evaluated in arena tests was reported by Peock and Maunder (1993).

Sulcatone was another major compound detected and the most volatile one. This ketone was previously reported as an important component of the upper back and forearm of human skin secretions using the same method of collection (Gallagher et al. 2008). Some blood-sucking arthropods are attracted by this compound, as *Cimex lectularis* (Linnaeus) (Hemiptera: Cimicidae) (Harraca et al. 2012) and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) (Logan et al. 2008), and others are repelled, as the Scottish biting midge *C. impunctatus* (Logan et al. 2009). In the present study, we found a downward trend in the proximity to the source at the highest masses, suggesting repellency (Fig. 3A). Probably, the high volatility of sulcatone produced a decrease in concentration of sulcatone along the time of the experiment.

Geranylacetone produced a similar response to sulcatone (Fig. 3C), showing low attractiveness at lower masses and low repellency at higher masses. It was previously demonstrated that this compound produced behavioral responses in host searching hematophagous insects, being an olfactory stimulant for *Anopheles gambie* (Linnaeus) (Diptera: Culicidae) (Meijerink et al. 2000) and a repellent for the Scottish biting midge (Logan et al., 2009).

Palmitic acid showed a different pattern of response, as it was more repellent at lower concentrations than at higher concentrations (Fig. 3). Different insect responses were described for the exposure to this acid, e.g., no response was detected in *A. gambiae* (Knols et al. 1997) and a significant response was described in *Ae. aegypti* and *Culex quinquefasciatus* (Say) (Diptera: Culicidae) compared to water controls controls (Allan et al. 2005).

We demonstrated significant response to volatile compounds extracted from the human scalp rather than to neutral odors, as well as to the individual main component nonanal. A similar trend in the response of head lice to sulcatone and geranylacetone was demonstrated, although a larger sample size should have been used to increase the power of the GLM and to detect significant differences between different masses. Another point of our study was that head lice show a similar response to pure compounds secreted by their potential hosts.

A significant finding of our study was that nonanal activity depends on its concentration, that is, repellent at high concentrations and attractant at low concentrations. In the next stage of our research, the effect of different volatiles mixtures will be evaluated to study chemical interactions like synergism and antagonism among them as was demonstrated for other insects (Barrozo et al. 2004, Logan et al. 2009). The results of this study indicate that head lice may use chemical signals in addition to other mechanisms to remain on the host.

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