Entomologia
Experimentalis et Applicata

DOI: 10.1111/eea.12297

A floral-derived attractant for *Aedes aegypti* mosquitoes

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Accepted: 5 February 2015

Key words: *Lobularia maritima*, acetophenone, imidacloprid, olfactometer, 1-octanol, *Tagetes patula*, *Plectranthus neochilus*, Diptera, Culicidae

Abstract

The reproductive success of *Aedes aegypti* (L.) (Diptera: Culicidae) is strongly dependent on the availability of carbohydrates in the environment and the ability of the mosquitoes to locate them. The most significant source of carbohydrates for mosquitoes is nectar from flowering plants, which mosquitoes locate by their volatile compounds. The aim of our work was to identify plant volatile compounds that elicit a behavioral response in *Ae. aegypti*, which may be included in a mosquito trap for surveillance and/or control purposes. Landing-preference bioassays were performed with plants of three species—*Plectranthus neochilus* Schltr. (Lamiaceae), *Tagetes patula* L. (Asteraceae), and *Lobularia maritima* (L.) Desv. (Brassicaceae)—as lures and toxic sugar baits as landing markers. Mosquitoes landed only on *L. maritima*. Freshly cut inflorescences of *L. maritima* elicited a positive flight response in both sexes of mosquitoes. The analysis of the compounds in the static head space of *L. maritima* was performed by solid phase microextraction (SPME). Of the single volatile compounds tested, acetophenone was attractive and 1-octanol caused a flight aversive response. These findings are relevant as there are no reported plant-derived compounds attractive to *A. aegypti*. As both the male and female mosquitoes sugar feed, traps baited with plant odors are able to lure the whole adult population, making it an interesting option for including in future mosquito surveillance traps.

Introduction

Aedes aegypti (L.) (Diptera: Culicidae) is the main vector of dengue and chikungunya fever in the Americas (Wilder-Smith & Gubler, 2008). The mosquito has a wide geographic distribution in tropical and sub-tropical areas, including Argentina (Gubler & Clark, 1995; Vezzani & Carbajo, 2008). Adult male and female mosquitoes rely on carbohydrates to maintain their energetic balance. Carbohydrate uptake and availability have a critical influence on mosquito fecundity, longevity, flight capacity, and host-seeking behavior (Nayar & Sauerman, 1971; Nayar & Sauerman, 1975; Klowden, 1986; Foster, 1995). Although *Ae. aegypti* is able to complete the gonotrophic cycle on blood feeding alone, its fecundity is compromised by a failure to ingest carbohydrates (Briegel, 1990). Nectar is the main sugar source for mosquitoes (Grimstad & DeFoliart, 1974), and regular feeding behavior on plant tissue has been proven for the three mosquito genera *Culex*, *Anopheles*, and *Aedes* (Diptera: Culicidae), but given little attention (Müller & Schlein, 2005).

Plants are the only food source of male mosquitoes (Clements, 2008). Nectar-seeking behavior starts shortly after emergence and female mosquitoes are drawn to nectar sources earlier than to warm-blooded hosts (Foster, 1995). Plant probing was observed for *Anopheles sergentii* (Theobald) (Abdel-Malek, 1964) and for two *Aedes* species (Magnarelli, 1978). Some nectar sources have been proven more attractive and more suitable for increasing life expectancy and fecundity than others (Grimstad & DeFoliart, 1974; Magnarelli, 1978; Gadawski & Smith, 1992; Manda et al., 2007; Müller et al., 2011). Mosquitoes and many other nectar feeders are known to be attracted to floral volatile compounds (Vargo & Foster, 1982; Dudareva & Pichersky, 2000; Jhumur et al., 2007; Otienoburu et al., 2012). Odors seem to be primarily responsible for long-range attraction, and visual cues become more relevant at a shorter range (Thornsteinson & Brust, 1962; Healy & Jepson, 1988; Jepson & Healy, 1988). In the case of *Ae. aegypti*, a recent review by Nyasembe & Torto (2014) about plant-derived semiochemicals for mosquitoes states that flower compounds have been identified and evaluated by

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electroantennography, but no further behavioral work on their role as attractants has been done.

In this study, we investigated the involvement of plant volatiles in the feeding attraction of mosquitoes. The aim was to identify plant volatile compounds that elicit behavior in *Ae. aegypti*. Such a compound or blend of compounds could then be included in a mosquito trap, for surveillance and/or control purposes. Current commercial mosquito traps are baited with carbon dioxide and vertebrate skin volatiles. They only attract blood-seeking females, and have no effect on the rest of the adult mosquito population (Burkett et al., 2002). Traps with plant volatile-based lures are capable of acting on the whole population, making them suitable for population surveillance. The viability of using an attractive compound in a toxic sugar bait with the purpose of mosquito population control has been proven (Qualls et al., 2014).

No information was available about local plant species attractive to *Ae. aegypti*, so we selected locally abundant plants based on reports for other Diptera. We tested sweet alyssum, *Lobularia maritima* (L.) Desv. (Brassicaceae), attractant for *Delia radicum* (L.) (Rännbäck, 2008), lobster flower, *Plectranthus neochilus* Schltr. (Lamiaceae), attractant for Bombyliidae (Stirton, 1977), and French marigold, *Tagetes patula* L. (Asteraceae), attractant for Diptera in general (Colley & Luna, 2000).

Materials and methods

Biological material

An insecticide-susceptible CIPEIN strain of *Ae. aegypti* was used. This colony originated from the Rockefeller strain from Venezuela and has been kept in the laboratory since 1996. Eggs were laid on wet filter paper and left for 48 h. They were then dehydrated at ambient temperature and stored for at least 30 days. When needed, they were rehydrated by placing them in dechlorinated water (500 eggs per 2 l of water) at 25 ± 2 °C. After 24 h, first instars were observed. Pupae were separated and placed in 250-ml plastic containers and allowed to hatch in 20 × 20 × 20-cm acrylic cages. Larvae were fed a mixture of ground rabbit pellets and yeast powder (80:20 wt:wt). Adults were offered water and a 10% sucrose solution ad libitum. Mosquito adults and larvae were kept in the same room at 25 ± 2 °C, 70 ± 5% r.h., and with a L12:D12 photoperiod (Seccacini et al., 2006). Twenty-four hours before the experiment, they were deprived of sugar, as starvation has shown to increase their response toward volatile cues of food sources (Vargo & Foster, 1982; Jhumur et al., 2006). We used 2- to 7-day-old adults of both sexes.

Plants

Lobularia maritima and *T. patula* were purchased at Viveros El Botánico (San Fernando, BA, Argentina) as needed, rinsed with water, and left at least 3 days outdoors before being used. Plant parts of *P. neochilus* were collected from a field inside of our institute's property. We only used plants deemed healthy and undamaged after visual inspection.

Chemicals

Acetone (>99.8%) and 1-octanol (>96%) were purchased from Merck (Darmstadt, Germany). Sucrose (>99%) was purchased from Anedra® (Tigre, BA, Argentina). Acetophenone (>99%) was purchased from Carlo Erba Reagenti (Rodano, MI, Italy). 2-phenylethanol (>99%) was purchased from Alfa Aesar (Ward Hill, MA, USA). Benzyl cyanide (>98%), benzyl isothiocyanate (>98%), and alkane-standards (C5-C30) (>99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Imidacloprid (94.3%) was supplied by Bayer (Leverkusen, Germany).

Landing-preference bioassay

The acute oral toxicity of imidacloprid on adult *Ae. aegypti* mosquitoes has not been reported previously. To determine the dose to use in the bioassay, the acute oral toxicity of imidacloprid on adults was determined following Allan (2011). One mg l^{-1} of imidacloprid in a 10% sucrose solution resulted in 100% mortality of both female and male mosquitoes at 24 h (S von Oppen, unpubl.), in agreement with mortality values reported for closely related species. For use in the bioassays the dose was increased to 10 mg l^{-1} (= 10 p.p.m.) in a 10% sucrose solution because of the rapid mortality observed in 2 h.

The imidacloprid added to the container with the plant material served as a feeding marker, as its mode of action is mainly through oral ingestion, with little contact and inhalation toxicity (Tomizawa & Casida, 2005). Another advantage is that it causes no measurable spatial repellency nor does it elicit attraction behavior per se in *Ae. aegypti* (Antonio-Arreola et al., 2011). Sucrose has been proven to be a successful phagostimulant in insecticide-sugar solutions in mosquitoes (Xue & Barnard, 2003; Müller et al., 2010; Allan, 2011; Shin et al., 2011), so we soaked cotton plugs with a 10% sucrose solution and placed them on the surface of the container.

The assays were performed in 40 × 30 × 40-cm acrylic cages at 27 ± 1 °C, 80% r.h., and L12:D12. In each cage, a 125-ml high-density polyethylene (HDPE) container with 2 g of water-soaked cotton was placed in the middle along one side, and in the middle along two opposing sides, two 250-ml HDPE containers were located (Figure 1). One was filled with plant material and

covered with nylon cloth on which was placed a 0.25-g piece of cotton, soaked in a solution of 10 p.p.m. imidacloprid in 10% sucrose. The other container was left empty and covered with nylon cloth on which was placed a 0.25-g piece of cotton, soaked in a 10% sucrose solution. As a control, the setup was repeated, but without the plant material inside. Once the cages were set up, female and male mosquitoes were introduced (n = 7–12 of each gender). After 24 h, dead or knocked-down mosquitoes were removed, sexed, and counted to determine the percent mortality.

We also performed a control assay for correction of mortality, one per replica. Ten mosquitoes of each gender were released in a nylon cloth-covered 1-l HDPE container with 0.25 g of cotton soaked in a 10% sucrose solution at the bottom. Mortality was corrected by Abbott's formula (Abbott, 1925).

The plant parts tested were *L. maritima* inflorescences, *P. neochilus* vegetative tissue, and *T. patula* flowers. The amount of plant material needed to fill the 250-ml container used was 2, 10, and 5 g, respectively. Eleven replicates for each plant were done. We are aware that fragmenting the plant leads to changes in the volatile emission pattern, but the aim of this work was to find a plant-derived attractant, not the precise volatile composition of the tested plants.

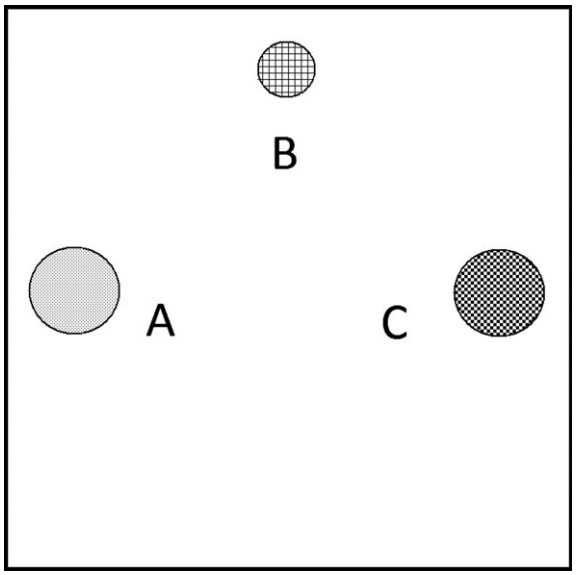

Figure 1 Cage setup for landing-preference bioassay. Circles represent 125-ml containers, placed on the bottom of a 40 × 30 × 40-cm cage. A, plant material + 10 p.p.m. imidacloprid in 10% sucrose solution, B: water C: 10% sucrose solution. The same setup was used as a control, without the plant material in container B.

Olfactometer behavioral assay

The Y-tube olfactometer was designed according to Geier & Boeckh (1999). The laboratory's central air supply provided an 80 l per min airflow through the olfactometer. Air was filtered through activated charcoal and adjusted to 70% r.h., by passing the air through distilled water at 20 °C and allowing it to reach equilibrium with the room's temperature of 26 °C while flowing through a 2-m-long hose. Experiments were performed between 10:00 and 18:00 hours. Latex gloves were worn when handling the mosquitoes, the plants, and the olfactometer. Before the start of an experimental session, the olfactometer was thoroughly cleaned with a cloth soaked with 96% ethanol. Watch glasses and plant material were held with tweezers at all times. After use, watch glass were rinsed with ethanol and then heated to 175 °C for 1 h.

Aedes aegypti flight preference to *L. maritima* was tested with 2 g of cut inflorescences set in a container connected to the air supply of one of the arms of the olfactometer, while plain filtered air flowed through the other arm. Plant material was renewed after five replicas or 2 h after being cut, whichever came first. A control assay was performed by measuring the mosquitoes' response to plain air in both arms of the olfactometer.

To test the flight preference toward single synthetic compounds identified in *L. maritima*'s head space, 100 µl of a 1-mg ml^{-1} acetone stock solution was put on a watch glass at the air entrance of one of the arms, for each replicate. A total of 100 µl of acetone was used as a blank in the opposite arm. The control of the volatile compounds' assay in the olfactometer was performed by measuring the mosquitoes' response to acetone in both arms. After the tested synthetic compound was in place, a mosquito was introduced at the base of the olfactometer. The airflow was opened and the mosquito was exposed to it for 2 min before being released. Only the mosquito's first choice of olfactometer arm was recorded. Unresponsive mosquitoes were discarded after 3 min. All the stimuli, inflorescences and single chemical compounds were placed in the left arm, to be able to recognize a statistical significant difference with the χ^2 test. The blanks of acetone and plain air were determined with 80 ± 4 responsive mosquitoes. Behavioral responses of mosquitoes to plant material and volatile compounds were tested with 40 ± 5 responsive mosquitoes.

Headspace analysis

Lobularia maritima's inflorescence static headspace (SHS) volatile profile was characterized by placing 1 g of freshly cut inflorescence into a 10-ml borosilicate vial, sealed with a Teflon-lined rubber septum. Headspace volatiles were allowed to reach equilibrium for 30 min before being

collected at 30 °C in a temperature-controlled system with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm) solid phase microextraction (SPME) fiber (Supelco, Bellefonte, PA, USA). Volatile collection was performed by puncturing the fiber-containing needle through the septum and exposing it for 30 min, followed by analysis in a Shimadzu QP-5050 gas chromatograph-mass spectrometer (GC-MS). Desorption of the SPME fiber was carried out at 240 °C for 1 min in the injector set to splitless mode. Before each volatile collection, a blank with the empty fiber was performed. Volatiles were analyzed with electron ionization (n = 3) in each of the following columns: DB-5MS (30 m × 0.25 mm, 0.25 μm film thickness), DB-Wax (30 m × 0.32 mm, 0.25 μm film thickness), and CYCLOSIL-B (30 m × 0.25 mm, 0.25 μm film thickness) of Agilent Technologies (Santa Clara, CA, USA) for chiral analysis. Volatiles were also analyzed using chemical ionization (CI) with methane as the reagent gas (3×), but only in the DB-5MS column. The oven was programmed at 50 °C for 2 min, then increased at a rate of 7 °C per min to 200 °C and held for 5 min. The carrier gas (He) had a flow rate of 2.1 ml per min. The detector was operated at 70 eV, scanning from 45 to 280 *m/z*, with an interface temperature of 245 °C. The chemical characterization of single volatile compounds was carried out, according to availability, by using a standard reference sample, 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).

Statistical analysis

The statistical analysis was performed with the InfoStat program package (InfoStat Group, 2008). The percent mortality, after correction, was compared by Student's t-test in the landing bioassay; the χ^2 test was used to evaluate the flight preference response in the olfactometer bioassay. The threshold for significance was $\alpha = 0.05$.

Results

Landing-preference bioassay response

As can be seen in Figure 2, *L. maritima* elicited a significant positive response in male (t = 3.38), and female (t = 2.40, both d.f. = 20, P<0.05) *Ae. aegypti*. However, *P. neochilus* and *T. patula* were not attractive to males (t = −1.11 and 0.10, respectively) nor females (t = −0.63 and 0.46; all d.f. = 20, P>0.05).

Olfactometer bioassay

Considering the previous results obtained in the landing-preference test, a dual-choice olfactometer was used to confirm the attractance of *L. maritima* to *Ae. aegypti*. The mosquitoes were found to prefer the airstream containing the volatiles of freshly cut *L. maritima* inflorescences, compared to the control arm (females: $\chi^2 = 5.40$; males: $\chi^2 = 5.05$; both d.f. = 1, P<0.05; Figure 3). Fewer than 10% of the mosquitoes were non-responsive.

Plant volatile identification

Acetophenone, 1-octanol, 2-phenylethanol, benzyl cyanide, and benzyl isothiocyanate were identified from the headspace of *L. maritima* by direct comparison with analytical standards, whereas 3-butenyl isothiocyanate, phenylacetaldehyde, 1,2-thiiranpentylnitrile, 4-methyl-pentyl isothiocyanate, hexyl isothiocyanate, and 4-vinyl-2-methoxy phenol were identified by comparing their MS and RI with literature data, because of the unavailability of synthetic standards (Table 1). 4-Pentenyl isothiocyanate was tentatively identified based on the comparison of the experimental RI with the bibliography and the interpretation of the MS. Lastly, 1,2-thiiranhexylnitrile was tentatively identified based on the MS interpretation and on older literature data (Cole, 1976; Daxenbichler et al., 1991) indicating its presence in *L. maritima*, as neither the RI nor an analytical standard were available.

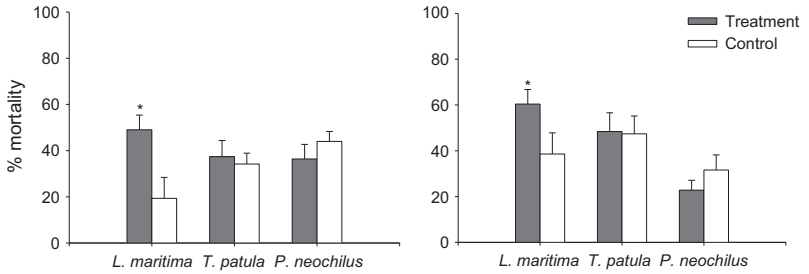

Figure 2 Behavioral responses of female (left) and male (right) *Aedes aegypti* in the plant choice landing bioassay. Preference for imidacloprid-sucrose baited plant material (2 g; treatment) was measured by comparing mean (+ SE) mosquito mortality against the same setup without the plant material (control). Asterisks denote a significant difference in response between treatment and control (Student's t-test: P<0.05).

An unknown compound was also found (RI = 1 395 in DB-5, 1490 in DB-wax). The molecular mass of the ion was confirmed to be 204 *m/z* through chemical ionization MS. Its mass, RI, and the mass-fragmentation pattern suggest a compound of terpenoid nature (Table 1).

Olfactometer flight preference bioassay with *Lobularia maritima* volatiles

Headspace volatiles identified in *L. maritima* inflorescences and available as synthetic compounds were tested in the olfactometer. Females displayed a positive response to acetophenone (χ^2 = 7.84, d.f. = 1, P<0.01; Figure 4). No effect on flight behavior was recorded for benzyl cyanide (χ^2 = 0.41), benzyl isothiocyanate (χ^2 = 0.002), and 2-phenylethanol (χ^2 = 0.075; all d.f. = 1, P>0.05). The presence of 1-octanol elicited a negative flight response (χ^2 = 4.84, d.f. = 1, P<0.05).

A positive flight response was also observed in male *Ae. aegypti* to acetophenone (χ^2 = 6.65, d.f. = 1, P<0.01). No effect on male flight behavior was recorded for benzyl cyanide (χ^2 = 2.04), benzyl isothiocyanate (χ^2 = 2.04), 2-phenylethanol (χ^2 = 0.26), and 1-octanol (χ^2 = 2.03; all d.f. = 1, P>0.05). Fewer than 10% of the mosquitoes were non-responsive.

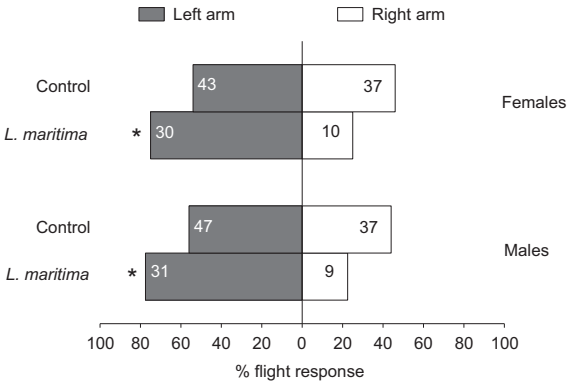

Figure 3 Choice response of *Aedes aegypti* in a dual-port flight olfactometer toward 2 g of freshly cut *Lobularia maritima* inflorescences, with no visual contact. Only responding mosquitoes that flew into one of the two arms were counted. Each horizontal bar marks the flight preference for an arm. An air stream laden with the plant volatile was tested in the left arm. Asterisks denote a significant difference in response between treatment and acetone control (χ^2 test: *P<0.05).

Discussion

Plants emit volatile organic compounds from different organs, such as flowers, fruits, and leaves. As plant sugar is an essential part of the mosquito diet and as it is the only food source for males, this study focused on the identification of attractive compounds elicited from floral and non-floral parts of ornamental plants. We found that the intact *L. maritima* plant was attractive, as has also been found for other insect species (Johanowicz & Mitchell, 2000; Sivinski et al., 2006; Rohrig et al., 2008a). *Plectranthus neochilus* and *T. patula* had no apparent effect on mosquito landing behavior. Although larvicidal activity of *T. patula*'s essential oil against *Ae. aegypti* has been reported (Dharmagadda et al., 2005), no repellence or attractance has been observed for extracts, plant parts, or whole plants.

Previous studies of *L. maritima* volatiles using a dynamic headspace technique (Rohrig et al., 2008b), found only acetophenone present both in whole plant and cut inflorescences. Our study reports several

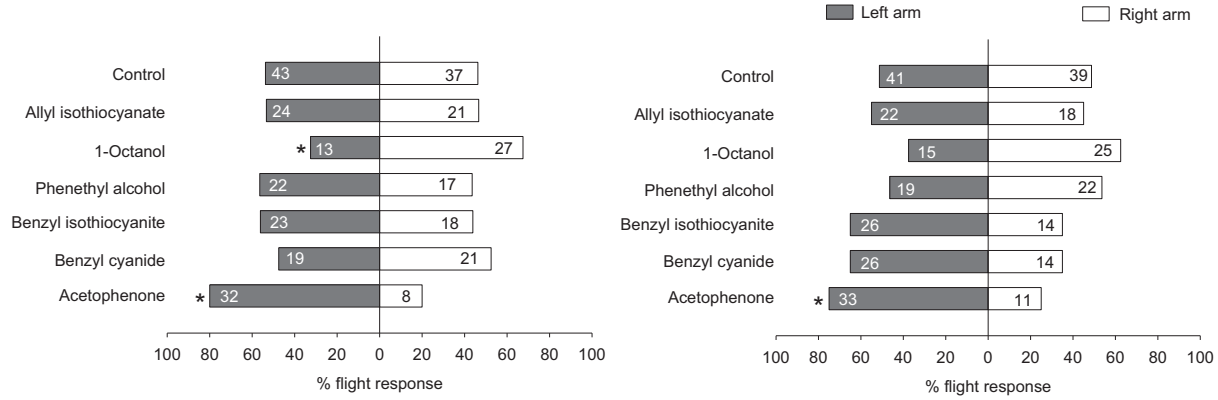

Figure 4 Behavioral responses of female and male *Aedes aegypti* in a dual-port flight olfactometer to six identified *Lobularia maritima* volatile compounds. Only responding mosquitoes that crossed the mark at the end of an arm were counted. Horizontal bars mark the flight preference for each arm in the presence of the substance tested. Compounds were offered in the left arm. Asterisks denote a significant difference in response between treatment and acetone control (χ^2 test: *P<0.05).

Table 1 Static headspace profile of *Lobularia maritima* inflorescences volatile compounds, collected by SPME in a DVB/CAR/PDMS fiber and analyzed by GC-MS in a DB-5 column

Retention time (min)	Relative RI	RI literature	Diagnostic EI-MS ions (% intensity)	Compound name	Identification method	Reference
6.92	981	973	113(M^+, 50), 72(M – C_3H_5, 100), 55(M – NCS, 31)	3-Butenyl isothiocyanate	a,c	Miyazawa et al. (2005)
8.41	1045	1044	120(M^+, 20), 91(M – HCO, 100), 65(M – HCO – C_2H_2, 28)	Phenylacetaldehyde	a,c	Lucero et al. (2006)
8.91	1067	1078	120(M^+, 35), 105(M – CH_3, 100), 77(M – CH_3 – CO, 99), 51(M – CH_3 – CO – C_2H_2, 58)	Acetophenone	a,b,c	Buchin et al. (2002)
8.98	1070	1078	130(M^+, 0), 112(M – H_2O, 1), 97(M – CH_3 – H_2O, 2), 56(C_4H_8, 100)	1-Octanol	a,b,c	Ramarathnam et al. (1993)
9.26	1081	1075	127(M^+, 51), 99(M – C_2H_4, 54), 72(M – C_4H_7, 72), 67(M – SH – HCN, 100)	4-Pentenyl isothiocyanate	a,d	Miyazawa et al. (2005)
10.01	1114	1120	122(M^+, 24), 91(C_7H_7, 100), 77(C_6H_5, 6)	2-Phenylethanol	a,b,c	Mahattanatawee et al. (2007)
10.28	1124	(HP5-1124)	113(M^+, 60), 98(M – CH_3, 3), 86(M – HCN, 15), 80(M – SH, 10), 45(CHS, 100)	1,2-Thiiranpentylnitrile	a,c	Afsharypuor & Suleimany (2002)
10.62	1140	1160	117(M^+, 100), 90(C_7H_6, 62), 77(C_6H_5, 9)	Benzyl cyanide	a,b,c	Rout et al. (2007)
11.07	1161	(HP5-1166)	143(M^+, 62), 128(M – CH_3, 82), 110(M – SH, 52), 72(M – C_5H_{11},72), 56 (C_4H_8, 100)	4-Methylpentyl isothiocyanate	a,c	Afsharypuor & Suleimany (2002)
11.91	1197	(HP5-1199)	143(M^+, 5), 115(M – C_2H_4, 100), 110 (M – SH, 30), 72(M – C_5H_{11}, 52)	Hexyl isothiocyanate	a,c	Afsharypuor & Suleimany (2002)
12.93	1245		127(M^+, 38), 112(M – CH_3, 3), 100(M – HCN, 8), 94(M – SH, 21), 45(CHS^+, 100)	1,2-Thiiranhexylnitrile	d	
14.37	1312	1311	150(M^+, 100), 135(M – CH_3, 93), 107 (M – C_2H_2 – OH, 63), 77(M – H_2CO – C_2H_2 – OH, 70)	4-Vinyl 2-methoxy phenol	a,c	Scheidig et al. (2007)
15.45	1365	1353	149(M^+, 22), 91(C_7H_7, 100), 65(C_5H_6, 25)	Benzyl isothiocyanate	a,b,c	Miyazawa et al. (2005)
15.95	1390		204(M^+, 7), 134(100), 119(39), 105 (83), 91 (22), 77(6)	Unknown		

Identification methods: (a) retention index (RI) from the literature; (b) standard injection of reference sample; (c) comparison of mass spectrum to a database; (d) interpretation of mass spectrum.

additional compounds and represents a contribution to the characterization of the *L. maritima* volatiles profile. Many of the identified compounds in *L. maritima* headspace are in line with the ones previously reported in the extracts of this plant (Cole, 1975; Bennett et al., 2004). Isothiocyanates, nitriles, and thiiran nitriles, which were tentatively identified, are known products of the hydrolysis of glucosinolates, by enzymes released after mechanical damage in plants of the Brassicaceae family to which *L. maritima* belongs (Halkier & Gershenzon, 2006). The presence of 1,2-thiiranpentylnitrile and 1,2-thiiranhexylnitrile was expected, as they are the result of the sulfur rearrangement of 3-butenyl-isothiocyanate and 3-pentenylisothiocyanate, respectively. Another hint that reinforces their identity is that in the analyses performed on a chiral column, we observed two consecutive peaks with identical mass spectra and it is clear that both possible enantiomers could have formed during the sulfur rearrangements.

We tested synthetic acetophenone, 1-octanol, 2-phenyl ethanol, benzyl cyanide, and benzyl isothiocyanate for attractance. Only acetophenone elicited a positive flight behavior in male and female mosquitoes, whereas 1-octanol showed a negative flight response in female *Ae. aegypti*. Acetophenone is the first plant-derived compound that has been shown to elicit a positive flight behavior in *Ae. aegypti*, as previous works on floral compounds only evaluated electroantennogram recordings of potential floral attractants (Nyasembe & Torto, 2014). However, Jhumur et al. (2007) did find attraction of female *Culex pipiens* L. to acetophenone cues in a wind tunnel. Acetophenone and 2-phenyl ethanol have been reported to elicit EAG signals in *Ae. aegypti* (Jhumur et al., 2007), but no behavioral bioassays were performed.

The fact that no statistically significant behavior in the olfactometer was found for 2-phenyl ethanol could be attributed to the concentration evaluated, which may not have been adequate to reveal a behavioral response, or perhaps it only enhances the effect of other volatile compounds when delivered in a blend, or it may have a behavioral effect but not detectable in our flight behavior assay. Mauer & Rowley (1999) found that *C. pipiens* were attracted in a dual port to common milkweed flower extracts. Its headspace volatile profile was predominantly composed of 2-phenyl ethanol and benzyl alcohol. However, a synthetic blend of these two compounds was not found to be attractive (Mauer & Rowley, 1999).

The flight aversion response to 1-octanol by the female mosquito suggests repellent properties at the dose tested. There are no literature data of any behavioral effect of 1-octanol, or blends containing it, on *Ae. aegypti*. As the applied methodology is not optimal for testing repellency, further studies will be needed to understand its influence on the behavior of mosquitoes.

Our result is interesting for the objective of finding natural attractants to add to mosquito traps deployed in the field. The main advantage of this plant-based mosquito attractant is that it works on female as well as male mosquitoes. Most mosquito traps based on chemical lures are based on carbon dioxide and 3-octenol and are only effective for blood-seeking females, leaving a broad spectrum of the mosquito adult population unaffected (Takken & Kline, 1989; Canyon & Hii, 1997). The use of compounds such as acetophenone and other plant volatiles showing similar effects in *Ae. aegypti* could be a promising tool to optimize adult trap devices, leading to improved monitoring and control systems in endemic areas.

Acknowledgements

This study received financial support by the ANPCyT (Agencia Nacional de Promoción de la Ciencia y la Tecnología) of Argentina (PICT 2008-0797). PGA, HM, and EZ are members of the CONICET (National Research Council of Argentina) and of University of San Martin (UNSAM). SO has a grant from CONICET.

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