J Chem Ecol (2008) 34:14–25
DOI 10.1007/s10886-007-9392-0

Floral Odors of *Silene otites*: Their Variability and Attractiveness to Mosquitoes

Umma Salma Jhumur · Stefan Dötterl · Andreas Jürgens

Received: 26 June 2007 / Revised: 7 September 2007 / Accepted: 19 October 2007 / Published online: 5 December 2007

Abstract Inflorescence scent samples from nine populations of dioecious *Silene otites*, a plant pollinated by moths and mosquitoes, were collected by dynamic headspace extraction. Sixty-three scent samples were analyzed by gas chromatography–mass spectrometry. Out of 38 found, 35 compounds were identified, most of which were monoterpenoids, fatty acid derivatives, and benzenoids. Phenyl acetaldehyde was the most dominant compound in the majority of samples. The variability in scent composition was high, and population and sex differences were found. Nevertheless, wind tunnel experiments proved similar attraction of *Culex pipiens pipiens* biotype *molestus* mosquitoes to the inflorescence odor of *S. otites* of different populations, indicating that different blends are similarly attractive to mosquitoes. The electrophysiological responses of mosquitoes to the 12 most common and abundant odor compounds of *S. otites* differed. Linalool oxide (furanoid) and linalool evoked the strongest responses in male and female mosquitoes, and (*Z*)-3-hexenyl acetate was strongly active in females. Medium responses were evoked in males by (*Z*)-3-hexenyl acetate, in females by benzaldehyde and methyl salicylate, and in both sexes by lilac aldehyde, lilac alcohol, and linalool oxide (pyranoid).

Keywords *Silene otites* · Flower odor variability · Wind tunnel bioassays · *Culex pipiens pipiens* biotype *molestus* · Electroantennography · Attraction · Nectar host plant

U. S. Jhumur (✉) · S. Dötterl
Department of Plant Systematics, University of Bayreuth,
95440 Bayreuth, Germany
e-mail: jhumurjb@yahoo.com

A. Jürgens
School of Botany and Zoology, University of Natal,
P. Bag XO I, Scottsville,
Pietermaritzburg 3209, South Africa

Introduction

Carbohydrates are vital resources for adult male and female mosquitoes. Uptake of sugar plays a critical role in longevity, fecundity, flight capacity, and host-seeking behavior (Harada et al. 1971; Nayar and Saurman 1971, 1975; Magnarelli 1978; Klowden 1986). The primary sugar source for mosquitoes is nectar (Haeger 1955; Sandholm and Price 1962; Grimstad and DeFoliart 1974), and mosquitoes prefer some plants to others as nectar sources (Grimstad and DeFoliart 1974; Magnarelli 1978; Gadawaski and Smith 1992). However, the specific cues that mosquitoes use to find and to select nectar sources are not well understood. Many flower visitors, mosquitoes included, are known to be attracted to floral scents (Vargo and Foster 1982; Dudareva and Pichersky 2000).

For finding effective nectar-related attractants for biological control of mosquitoes, it is important to determine which plant species produce the most attractive floral compounds and to identify these compounds. Plant species adapted to mosquitoes as pollinators are expected to emit more mosquito-attracting compounds than plants pollinated primarily by other pollen vectors.

Worldwide, effective pollination by mosquitoes has been described only in the orchid *Habenaria (Platanthera) obtusata* (Banks ex Pursh) Richardson (Stoutamire 1968) and in *Silene otites* L. Wibel (Caryophyllaceae) (Brantjes and Leemans 1976), which is usually a perennial and dioecious species widely distributed in Middle, East, and South Europe and in Central Asia. The small and white-greenish flowers are arranged in terminal cymes. Jürgens et al. (2002) described the floral scent composition of *S. otites*. The scent of a few plants of a single *S. otites* population was analyzed. Therefore, nothing is known about the variability in the scent of this plant among populations or between males and females.

Compounds with low variability may be more important for the attraction of pollinators than compounds with high variability (Ayasse at al. 2000), as pollinators may exert selective pressure on scent composition, resulting in regular emission of attractive compounds, whereas nonattractive compounds may be more variable. So far, only a single major volatile component of *S. otites*, phenyl acetaldehyde, has been shown to attract mosquitoes (Jhumur et al. 2006), whereas the importance of the total floral scent emitted by *S. otites* is unknown for attraction of its flower-visiting mosquitoes (e.g., *Culex pipiens* L. and *Culiseta annulata* Schrank; Brantjes and Leemans 1976).

The aim of this study was to analyze the geographic variability of the floral scent composition of *S. otites* (L.) Wibel (Caryophyllaceae), and to assess the attractiveness of floral bouquets of different *S. otites* populations to *Culex pipiens pipiens* biotype *molestus* Forskal 1775. Furthermore, the antennal electrophysiological responses of *C. p. molestus* to the most common and abundant odor compounds in *S. otites* were measured.

Methods and Materials

Plant Material Inflorescence scent samples were collected from 63 individuals of 9 different populations. The geographic origin of eight populations and the number of females and males sampled are shown in Fig. 1. For one population (h), from which three males and four females were sampled, the geographic origin is unknown. Seed of the different populations were provided by several botanical gardens. To reduce environmental variation among populations, plants were grown under the same conditions (e.g., soil, temperature) in pots in the greenhouse until they built up a rosette, and thereafter the pots were placed in flower beds in the field.

Odor Collection S. otites is a nocturnal plant. Its floral scent emission is strongest in the early night hours (Jürgens et al. 2002). Male flowers remain functional for two nights, whereas female flowers emit scent over several days until they are pollinated (Brantjes and Leemans 1976). For studying the variability of floral scents, floral odors of *S. otites* were collected from one to four inflorescences of each individual plant 2–3 d after the onset of floral bloom when most of the flowers in an inflorescence had opened for the first time. Thus, the inflorescences used were of the same age; however, the flowers of these inflorescences were in different developmental stages. It is unclear whether there is variation in scent of *S. otites* among flowers of different ages on the same plant and whether this possible variation contributed to the observed variability among populations. However, as scent was collected from inflorescences of the same age, the possible variation in scent among flowers of different ages is not expected to have influenced our measurements. Furthermore, in a closely related species, *S. latifolia*, no differences in scent composition of flowers of different stages were found (Dötterl et al. 2005b).

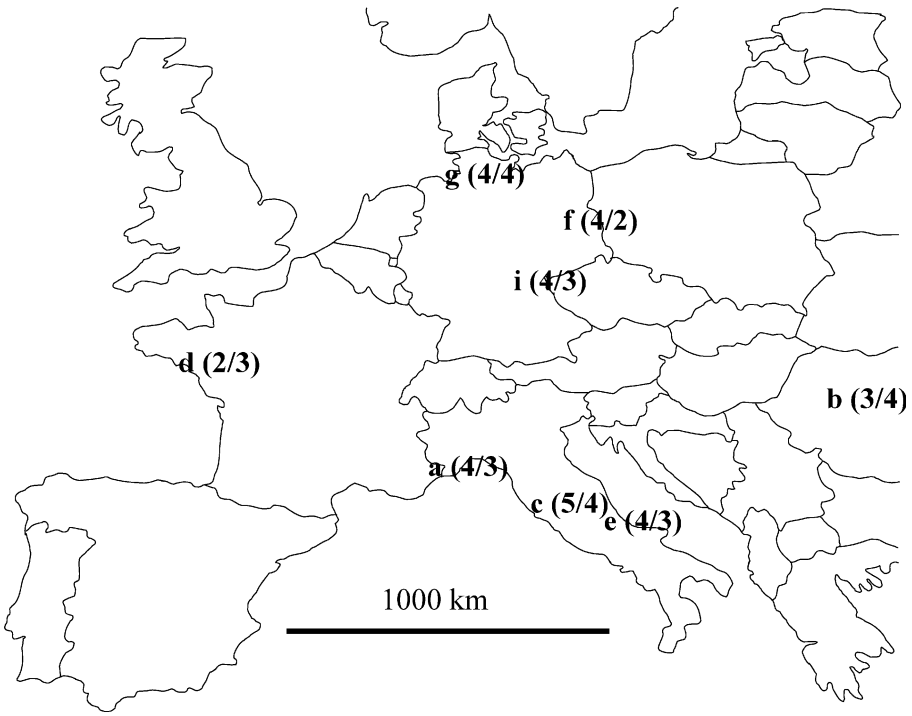

Fig. 1 Geographic origin of eight out of nine *S. otites* populations analyzed (a–f, i; the geographic origin of population 'h' is unknown). The number of sampled male and female individuals of each population is given in parenthesis

To collect odors, potted plants were placed under the extractor hood in the laboratory. Volatiles were collected by using the dynamic headspace method described by Dötterl et al. (2005b). Inflorescences were enclosed in a polyester oven bag (20×8 cm; Toppits®, Germany) 1–1 1/2 hr after sunset, and volatiles were trapped in an adsorbent tube for 2 min by using a membrane pump (ASF Thomas) with a flow rate of 200 ml/min. The adsorbent tubes were filled with a mixture (1:1) of 3 mg Tenax-TA (mesh 60–80) and Carbotrap (mesh 20–40). To distinguish between plant volatiles and ambient contaminants, surrounding air was collected for comparison. Furthermore, to discriminate odor emitted by flowers from odor derived from vegetative parts, scent was also collected from nonflowering shoots. However, as insects attracted to plants may detect green leaf volatiles and floral odors, we also included vegetative odors in subsequent analyses (see below).

Preparation of Plant Material for Bioassays To facilitate the work with the night-active plant-flower visitor system, plants were shifted from flower beds to a climatic chamber with an inverted day and night rhythm shortly before onset of flowering. Maintenance of the climatic chamber was dark (9 hr: from 9 A.M. to 6 P.M.) and light (15 hr: from 6 P.M. to 9 A.M.) with 20.5°C and 24.5°C, respectively. One or 2 d after moving, when flower opening had adjusted to the changed day and night rhythm, inflorescences were used for bioassays. Flower odors were collected before and after each bioassay, and are expressed as the mean total amount of emitted odors during bioassays. Flowering inflorescences (three to five) of males or females of a population were cut and placed together in small glass bottles filled with water. Within 5 min, inflorescences were bagged, and thereafter volatiles were collected for 2 min as described above. With the exception of higher amounts of green leaf odors in cut plants, the scent compositions of clock-shifted plants were the same as those of *in situ* plants (Jhumur, unpublished data).

Preparation of Insects for Bioassays We used flower-naïve individuals of the autogenous *Culex pipiens pipiens* biotype *molestus* Forskal 1775 (European strain) for experiments. Mosquitoes were reared according to Jhumur et al. (2006) with an inverted day and night rhythm in accordance with the designed bioassays. For bioassays, the sugar supply was removed 61–63 hr before the experiment. For electrophysiological measurements, regularly fed mosquitoes were used.

Chemical Scent Analysis Scent samples were analyzed on a Varian Saturn 2000 mass spectrometer coupled to a Varian 3800 gas chromatograph equipped with a 1079 injector that had been fitted with the ChromatoProbe kit. The adsorbent tube containing sample was placed in the Chromatoprobe and then inserted into the modified GC injector. The injector split vent was opened (1/20), and the injector was heated to 40°C to flush any air from the system. The split vent was closed after 2 min, and the injector was heated to 200°C (200° C/min); this temperature was held for 4.2 min. Then, the split vent was opened again (1/10) while the injector was cooled. For analyses, a ZB-5 column (5% phenyl polysiloxane; 60 m long, i.d. 0.25 mm, film thickness 0.25 μm, Phenomenex) was used. A constant flow of carrier gas (helium, 1.8 ml/min) was maintained by electronic flow control. The GC oven temperature was held for 7 min at 40°C, then increased by 6°C/min to 250°C, and held for 1 min. The MS interface was 260°C, and the ion trap worked at 175°C. The mass spectra were taken at 70 eV (in EI mode) with a scanning speed of 1 scan sec^{-1} from *m/z* 30 to 350. The GC–MS data were processed by using the Saturn Software package 5.2.1. Component identification was carried out with the NIST 02 mass spectral data base or MassFinder 2.3, and confirmed by comparison of retention times with published data (Adams 1995). Identification of individual components was confirmed by comparison of both mass spectra and GC retention data with those of authentic standards.

For quantification of odors emitted from inflorescences, known amounts of lilac aldehydes (>99%, synthesized according to Dötterl et al. 2006b), (Z/E)-β-ocimene (>99%, provided by Jette T. Knudsen, Lund University, Sweden), (*Z*)-3-hexenyl acetate, benzaldehyde, phenyl acetaldehyde, and veratrole (all purchased from Sigma-Aldrich with highest purity available) were injected into the GC for calibration.

Bioassays A 160×75×75-cm wind tunnel (Dötterl et al. 2006b; Jhumur et al. 2006) was used for bioassays. A Fischbach speed controller fan (D340/E1, FDR32, Neunkirchen, Germany) continuously circulated air through the tunnel with an air speed of 0.35 m/sec. Incoming air was cleaned through four charcoal filters (145×457 mm, carbon thickness 16 mm, Camfil Farr). To allow mosquitoes to adapt to the wind tunnel environment, they were kept in the wind tunnel room for about 12 hr before the experiment started. To avoid contamination, all equipment was cleaned with ethanol, burned in flame, and then sterilized at 200°C, and surgical gloves were worn during mosquito handling and bioassays.

At the conditions described above, *S. otites* emitted the highest amounts of floral odors in the 2nd and 3rd hr after onset of darkness (Jhumur, unpublished data). Therefore, bioassays were conducted within this time frame. The inflorescences, the cut ends of which were already inserted in water, were placed at the upwind end of the tunnel

behind gauze and different aluminum screens. They were invisible to the mosquitoes.

A group of 10–15 randomly chosen male and/or female mosquitoes (the behaviors of mosquitoes were not influenced by the opposite sex, see also Jhumur et al. 2006) were released from a chamber (16×8 cm) at the downwind end of the tunnel. Mosquitoes were observed for 1 hr. Landing on the gauze (20×10 cm) in front of the odor source was considered as attraction to the source. In addition, the latency time before landing was measured. After landing, the behavior was classified into two types: "sitting" and "searching". "Sitting" was characterized simply as sitting without moving or doing anything on the gauze for 15 sec after landing, and "searching" was characterized by excited movement of mosquitoes on the gauze and repeated penetration of gauze with their proboscis, presumably in search for a food source. To avoid recording the behavior of any responding mosquito twice, landing mosquitoes were removed from the wind tunnel after 15 sec with an aspirator.

From other tests with mosquitoes in the same wind tunnel, we know that almost no mosquitoes land just by chance in front of the odor source (Jhumur et al. 2006). Therefore, we did not test the mosquitoes' response to clean air or room air. Furthermore, given that a small number of mosquitoes would land just by chance on the gauze in front of the odor source, this number should be similar for odor from all *S. otites* populations, and thus, not affect the comparison of attractiveness of *S. otites* odor from different populations.

Dependent upon the availability of flowers, 25 bioassays were conducted with *S. otites* plants of six populations. Male and female inflorescences were tested separately. However, female inflorescences were not available for the 'a' and 'c' populations (Table 2). Nine bioassays were conducted with population 'i', six with 'f', four with 'g', and two each with 'c', 'b', and 'a'. Most of the inflorescences of one plant (one bioassay) were tested with two groups of mosquitoes, and the behavioral responses (percentage of individuals landing) of these 20–30 mosquitoes were used for subsequent statistical analyses (see below). However, for population 'a', only one group of mosquitoes was used for each of the two inflorescence samples. In total, 113 male and 531 female mosquitoes were tested. Male mosquitoes were not available during the bioassays with male inflorescences of populations 'a', 'c', and 'g', and female inflorescences of population 'b'.

Electrophysiology

Authentic Standard Compounds The most frequently found 12 floral scent compounds of *S. otites* were used for electrophysiological measurements. Among these, lilac aldehyde (purity >99%) was synthesized as described by Dötterl et al. (2006b); lilac alcohol and linalool were provided by Karlheinz Seifert (University Bayreuth, Germany; purity >99%); and the other compounds were purchased from Sigma-Aldrich (hexanol, (*Z*)-3-hexen-1-ol, and (*Z*)-3-hexenyl acetate >98%; benzaldehyde 99%; phenylethyl alcohol 99%, acetophenone 98%; linalool oxide [furanoid] 97%; phenyl acetaldehyde 90%; methyl salicylate 98%) or Wako (linalool oxide [pyranoid] 98%). Among these 12 compounds, all monoterpenoids were used as stereoisomeric mixtures. To obtain dose–response curves and to compare the sensitivity of mosquitoes to different compounds, electroantennographic (EAG) recordings were performed with a dilution series of standard compounds (Schütz et al. 1999). Dilutions were prepared in paraffin oil (Uvasol, MERCK, Darmstadt, Germany).

Preparation Four- to 5-d-old *C. p. molestus* were used for EAG. For measurements, an excised antenna was mounted between glass micropipette electrodes filled with insect ringer (8.0 g/l NaCl, 0.4 g/l KCl, 0.4 g/l $CaCl_2$). The electrodes were connected to silver wires. Signals were interfaced with a two-channel USB acquisition controller (provided by Syntech, Hilversum, The Netherlands) to a PC as described by Dötterl et al. (2005a). Twenty microliters of a test compound was placed onto a piece of filter paper (2.5×1.5 cm^2) inside a 5-ml plastic syringe (Omnifix, B/Braun, Melsungen). Separate syringes were used for each stimulus. Stimuli were released into a continuous flow of humidified air that passed over the antenna with a pulse duration of 0.5 sec, and a flow of 10 ml/sec regulated by a CS-01 Stimulus Controller (Syntech). Each compound and each dilution was tested on four to six mosquitoes. In all EAG tests, antennae were stimulated at 30–40 sec intervals. To discriminate between the antennal response elicited by the air flow or by paraffin and by the tested scent compound, a filter paper that contained only paraffin was tested as the first and last measurement on each antenna. To counterbalance for the loss of antennal sensitivity during measurements, the antennal response to a syringe containing (*Z*)-3-hexen-1-ol (10^{-1} in paraffin) was recorded as the second measurement from the beginning to the end. (*Z*)-3-Hexen-1-ol is a compound frequently found in sampling of *S. otites*. As this was used as the standard for EAG recordings, it was not used to obtain dose–response curves.

Statistical Analysis We used the Primer 6 program (Clarke and Warwick 2001; Clarke and Gorley 2006) to assess the variability in scent of *S. otites* individuals of different populations. Semiquantitative data of compounds (percentages=relative amounts with respect to total peak areas) were used because the total amount of emitted volatiles varied greatly among different individuals (see also Dötterl

et al. 2005b). We used multidimensional scaling (MDS) based on Bray–Curtis similarities to detect similarities among samples. To evaluate how well or poorly the particular configuration produces the observed distance matrix, the stress value is given. The smaller the stress value, the better the fit of the reproduced ordination to the observed distance matrix (Clarke 1993). We used ANOSIM (two-way crossed design, factors: sex, population) in Primer to test for the differences in scent between male and female flowers and among populations. SIMPER (two-way crossed design, factors: sex, population) was used in Primer to identify the compounds responsible for dissimilarities between sexes and among populations. RELATE was used in Primer to correlate the scent matrix with the distance matrix (in km) of the populations. To obtain the scent matrix, mean relative amounts of compounds were calculated for the different populations, and these values were used to calculate the Bray–Curtis similarities finally used for the analysis.

Chi-square tests were used to assess the differences in attractiveness between male and female mosquitoes (number of males responding–males not responding vs. number of females responding–females not responding) to male and female inflorescences of different populations of *S. otites*. No differences in responses between males and females were found (Jhumur, unpublished data). Therefore, the responses of males and females were pooled for further analyses.

In individual bioassays with specific inflorescences, the number of landing (attractive) mosquitoes (%) was determined at first, and among the landed mosquitoes thereafter the proportion of searching mosquitoes (%) was calculated. Kruskal–Wallis–ANOVA followed by the Tukey–Kramer post hoc test for nonparametric data in STATISTICA (StatSoft 2004) was used to compare these behavioral responses to the flower odors of different populations. ANOVA was used to compare the latency time of individual mosquitoes to different populations. Normality was tested with the Kolmogorov–Smirnov test; homogeneity of variances was tested by using the Hartley test.

For analyzing the EAG recordings, at first, the responses from the blank syringes were measured and subtracted from the recordings in between. Then, the response to (*Z*)-3-hexen-1-ol as the second measurement from the beginning of each measurement was set to 100%. As the sensitivity of antennae decreased during measurements, the response to (*Z*)-3-hexen-1-ol was also measured as the second measurement from the end, to determine the loss of sensitivity and to compensate for this. The responses to different compounds and dilutions are given as proportions of the responses to (*Z*)-3-hexen-1-ol (10^{-1} in paraffin). These data were directly used without transformation for further analyses. A general linear model (GLM) in STATISTICA was used to compare the differences in the responses of males and females to different dilutions and different compounds. The α-level for all statistical analyses was 0.05.

Results

Variability in Floral Scents of S. otites Thirty-eight compounds were detected in the inflorescence odor samples of *S. otites* of 9 geographical locations, 35 of which were tentatively identified by comparing mass spectra and retention index with literature data (Adams 1995). In addition, the identity of 27 of these compounds was confirmed by authentic standards (see Table 1). Among these, six compounds were also emitted from leaves. The identified compounds belong to 5 classes: fatty acid derivatives (8), benzenoids (6), nitrogen-containing compounds (1), monoterpenoids (18), and sesquiterpenoids (2). The benzenoid phenyl acetaldehyde (PAA) was the dominant odor compound in most of the individuals. However, one specimen emitted no PAA but instead high relative amounts of lilac aldehyde. Out of the 38 compounds, 19 were common to the scent samples of all populations.

Semiquantitative differences in the odor samples based on Bray–Curtis similarities are shown in Fig. 2. Variation among samples was high with significant differences among the samples from different populations (within sexes; two-way ANOSIM: $R=0.454$; $P<0.001$). SIMPER analyses revealed the compounds responsible for the differences among populations. Most populations and samples were dominated by phenyl acetaldehyde, but in some samples, high relative amounts of lilac aldehyde (e.g., samples of population ‘g’) or (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate (e.g., samples of population ‘h’) were present. One sample of population ‘i’ was characterized by a high percentage (33%) of linalool. There was no correlation between scent and the distance matrix of the populations (RELATE: $\rho=-0.02$, $P=0.52$), indicating that populations close to each other were not more similar in their scents than distant populations.

Within populations, we found significant differences in scent between male and female plants (two-way ANOSIM: $R=0.129$; $P=0.038$). However, differences between males and females were less pronounced than the observed differences among populations. Within populations, both males and females emitted the same compounds, but the proportions of some differed between males and females. According to the SIMPER analysis, phenyl acetaldehyde and (*Z*)-3-hexenyl acetate were the main compounds responsible for the differences between males and females

Table 1 Relative amounts of compounds (mean±SE) in inflorescence odors of 63 *S. otites* plants from different populations

Compounds	a (7)	b (7)	c (9)	d (5)	e (7)	f (6)	g (8)	h (7)	i (7)
Fatty acid derivatives									
Hexanol[a]	0.36±0.16	0.14±0.03	0.13±0.05	0.15±0.06	0.12±0.04	0.19±0.06	0.26±0.22	0.04±0.02	0.28±0.08
(*Z*)-3-Hexen-1-ol[a,b]	3.64±1.92	6.32±1.75	2.33±1.25	1.83±0.47	3.39±2.22	7.21±2.09	3.2±1.69	15.01±6.01	8.74±1.71
(*E*)-3-Hexen-1-ol	0.53±0.16	0.14±0.06	0.4±0.19	0.41±0.22	0.36±0.35	0.19±0.08	0.01±0.01	0.23±0.08	0.45±0.14
(*Z*)-3-Hexenyl acetate[a,b]	4.27±1.84	10.33±2.73	1.85±0.81	5.36±1.89	8.12±4.1	6.95±2.65	5.31±2.68	15.18±5.39	7.26±1.64
(*E*)-2-Hexenyl acetate[a]	0.11±0.05	0.04±0.04	0.08±0.06	0.06±0.04	0.73±0.55	0.02±0.02	–	–	0.03±0.02
Hexyl acetate[a]	–	0.09±0.08	0.03±0.02	0.01±0.01	2.61±1.71	0.17±0.1	–	–	0.14±0.08
(*Z*)–3-Hexenyl butyrate[b]	–	0.23±0.08	0.01±0.01	0.08±0.07	–	0.35±0.17	0.01±0.01	0.05±0.03	0.33±0.14
(*E*)-4,8-Dimethyl 1,3,7 nonatriene[b]	–	0.01±0.01	0.15±0.08	–	–	–	–	–	–
Benzenoids									
Benzaldehyde [a,b]	4.62±0.67	7.26±1.37	2.08±0.37	7.1±1.02	4.05±1.18	8.26±1.74	6.67±1.26	2.59±0.73	5.12±1.08
Benzyl alcohol[b]	0.02±0.02	0.17±0.06	0.01±0.01	0.04±0.03	0.01±0.01	0.84±0.42	0.14±0.06	0.48±0.26	4.68±1.43
Phenyl acetaldehyde[b]	47.71±4.43	38.97±4.78	35.94±1.54	42.27±7.64	40.89±11.69	41.02±4.42	31.66±3.29	14.85±4.64	26.05±4.29
Acetophenone[b]	0.36±0.22	0.31±0.17	0.48±0.32	0.58±0.36	0.32±0.29	1.38±0.56	3.01±2.01	0.07±0.07	1.08±0.5
Phenylethyl alcohol[b]	0.82±0.25	5.18±0.74	1.82±0.47	2.02±0.72	1.87±0.83	5.31±0.68	1.25±0.38	1.44±0.65	6.98±0.96
Methyl salicylate[b]	0.07±0.03	3.27±1.16	0.16±0.06	0.03±0.03	–	0.41±0.17	0.03±0.03	2.36±1.32	0.07±0.04
N- bearing compounds									
3-Methyl-butyl-aldoxime (syn/anti)[b]	–	–	0.01±0.01	–	–	–	0.01±0	0.01±0.01	–
Monoterpenoids									
α-Pinene[b,c]	–	0.02±0.02	–	0.15±0.09	0.08±0.04	0.13±0.06	0.12±0.05	0.35±0.12	0.14±0.1
β-Pinene[b,c]	0.05±0.04	0.01±0.01	–	0.04±0.02	0.05±0.03	0.12±0.05	0.01±0.01	0.23±0.13	0.25±0.08
D-Limonene[b,c]	–	–	–	–	0.04±0.02	–	–	–	–
(*E*)-β-Ocimene[b]	–	–	tr	–	–	–	0.06±0.06	0.31±0.2	0.54±0.54
(*Z*)-Linalool oxide furanoid[b,c]	0.28±0.15	tr	0.87±0.21	–	0.78±0.52	0.02±0.02	0.01±0.01	–	0.16±0.04
(*E*)-Linalool oxide furanoid[b,c]	–	0.6±0.11	0.01±0.01	4.45±2.35	0.12±0.07	1.46±0.39	0.03±0.03	7.17±1.16	3.04±0.39
Linalool[b,d]	–	3.13±1.09	0.84±0.22	0.49±0.19	0.06±0.04	4.26±1.63	0.52±0.12	5.64±1.72	8.11±4.24
Hotrienol[c]	–	2.16±0.48	0.25±0.2	1.52±0.88	0.1±0.04	1.63±0.5	0.66±0.16	0.6±0.39	2.57±0.49
2,2,6-Trimethyl-2-vinyl-5-ketotetrahydropyran[c]	0.4±0.13	0.05±0.01	0.71±0.16	0.53±0.21	0.42±0.16	0.16±0.05	0.06±0.04	0.73±0.33	0.73±0.11
Lilac aldehyde A[b,d]	12.37±2.01	6.94±0.86	14.42±1.43	10.46±1.43	8.14±1.91	6.27±0.65	20.16±2.23	12.03±2.45	5.96±1.58
Lilac aldehyde B+C[b,d]	13.72±1.9	8.32±1.13	16.53±1.11	11.31±1.36	10.43±2.35	7.48±0.59	19.42±1.26	10.19±1.78	6.47±1.52
Lilac aldehyde D[b,d]	2.9±0.6	2.29±0.52	4.22±0.47	3.02±0.41	2.73±0.5	1.94±0.29	4.67±0.61	2.29±0.49	1.12±0.31
(*Z*)-Linalool oxide pyranoid[b,c]	5.99±2.97	0.33±0.13	10.11±4.99	0.23±0.22	8.71±4.95	0.76±0.23	1.47±0.95	0.43±0.17	0.84±0.19
(*E*)-Linalool oxide pyranoid[b,c]	0.66±0.3	0.59±0.21	4.36±1.21	6.74±2.08	3.83±1.58	2.45±0.66	0.1±0.05	5.28±2.3	5.76±1.02
Lilac alcohol A[b,d]	0.32±0.07	0.39±0.18	0.96±0.19	0.4±0.12	0.71±0.49	0.19±0.07	0.37±0.11	1.3±0.41	0.54±0.12
Lilac alcohol B+C[b,d]	0.36±0.08	0.49±0.13	1.03±0.18	0.51±0.15	0.94±0.68	0.36±0.1	0.59±0.15	0.81±0.27	0.7±0.16
Lilac alcohol D[b,d]	0.03±0.02	0.13±0.04	0.2±0.04	0.04±0.02	0.22±0.17	0.05±0.03	0.11±0.03	0.05±0.03	0.07±0.02
1-Hydroxy linalool[c]	–	1.55±0.49	–	0.04±0.02	–	0.29±0.28	0.05±0.05	–	1.79±0.88
Monoterpenoid 43, 67, 79, 91, 105, 121	–	0.01±0.01	–	0.08±0.06	0.09±0.06	0.05±0.03	0.01±0.01	0.2±0.06	0.01±0.01
Monoterpene oxide 39, 65, 79,91,105, 121, 135, 150	–	0.01±0.01	–	0.03±0.03	0.02±0.02	0.06±0.06	–	–	0.01±0.01
Sesquiterpenoids									
(*E*)-β-Caryophyllene[b]	–	0.1±0.1	–	0.02±0.01	0.04±0.02	0.03±0.02	–	0.06±0.06	–
Geranyl isovalerate	0.38±0.25	0.38±0.38	0.02±0.02	–	–	–	–	–	–
Unknown									
43, 67, 93, 109, 123, 151	0.04±0.02	0.04±0.02	–	–	–	–	–	–	–

The number of individuals sampled in each population (a–i) is given in parenthesis.
[a] Compounds were also found in samples collected from leaves.
[b] Compounds were identified by comparing mass spectra and retention times with authentic standards.
[c] Enantiomeric composition was not determined
[c] Enantiomeric composition was determined by Dötterl et al. (2006a).

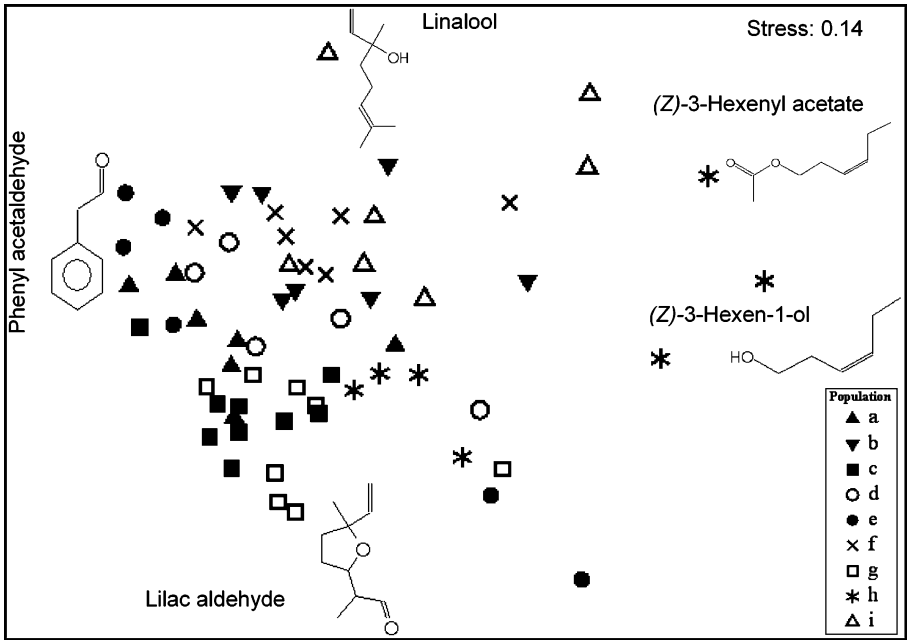

Fig. 2 MDS based on Bray–Curtis similarities of the odor composition of 63 inflorescences from 9 populations of *S. otites*. Most of the samples were dominated by high relative amounts of phenyl acetaldehyde; however, in some samples, high relative amounts of other compounds, such as lilac aldehyde, were found

(PAA=38% in males, 31% in females; (*Z*)-3-hexenyl acetate=8% in females, 6% in males).

Behavioral Responses of Mosquitoes to Odors of S. otites Inflorescences The wind tunnel bioassays revealed that about 50% of tested mosquitoes were attracted to scents emitted from inflorescences of *S. otites* of different populations. Male and female inflorescences were equally attractive to mosquitoes ('b': *Chi-square test*: $\phi^2_{df=1}=0.25$, $P=0.62$; 'f': $\phi^2_{df=1}=0.03$, $P=0.87$; 'g': $\phi^2_{df=1}=1.26$, $P=0.26$; 'i': $\phi^2_{df=1}=1.74$, $P=0.19$). Therefore, the responses to female and male inflorescences were pooled for further analyses. No differences in attractiveness among populations were found (Kruskal–Wallis–ANOVA: H (5, 25)=4.3; $P=0.5$). There was high variability in attraction within populations, which could not be explained by the different total amounts of scent emitted (Table 2, Fig. 3). As an example, most inflorescences of populations 'i' emitted similar total amounts of floral scent, but their attractiveness differed strongly (34–73%).

The latency time of mosquitoes did not differ among populations (ANOVA: F (5, 314)=0.33; $P=0.89$), and was on average 30 min. However, overall significant differences were found in the post choice behavior (Kruskal–Wallis–ANOVA: H (5, 25)=11.139; $P=0.049$). The 'searching' behavior was recorded most often when inflorescences of population 'a' were offered to the mosquitoes, and less often when they were offered inflorescences of population 'c'. Nevertheless, there were no significant differences in post hoc tests.

Electrophysiological Recordings EAG responses of male and female *C. p. molestus* to several odor components of *S. otites* are shown in Fig. 4. All tested compounds elicited EAG responses, and the effect of dilution was evident for each compound. EAG responses generally increased with increasing dose of tested compounds. However, mosquitoes responded differently to compounds tested, and we also recorded differences in the responses of males and females to different compounds (Table 3). The strongest responses (110–151%) were elicited by linalool oxide (furanoid) and linalool. Furthermore, females responded strongly to (*Z*)-3-hexenyl acetate. Weak responses (<80%) were obtained from both sexes to phenyl acetaldehyde, phenylethyl alcohol, acetophenone, and hexanol.

Discussion

Most compounds found in this study have been reported earlier as part of the floral odor bouquet in other angiosperms (Knudsen et al. 2006), but only nine of the compounds identified in this study were also found in the *S. otites* samples analyzed by Jürgens et al. (2002). In total, we found 22 new compounds in the floral scent of *S. otites* that have not been reported previously in that species. On the other hand, Jürgens et al. (2002) identified nine compounds that were not detectable in our samples. Furthermore, only small amounts of phenyl acetaldehyde were found in that study, but we found that this was the dominant compound in nine populations. Some of these differences might be ascribed to different scent collection

Table 2 Attraction, post choice behavior, and latency time of mosquitoes with respect to the emitted scent from *S. otites* inflorescences (three to five) of different populations (a–i)

Population (Numbers of Female (F) and Male (M) Inflorescence Samples Tested in Bioassays)	Number of Mosquitoes Tested	Odor Emission of Inflorescence Samples (ng/2 min) Median (Min–Max)	Number of Landed (Attracted) Mosquitoes (%) Median (Min–Max)[a]	Number of Landed Mosquitoes Showing Searching Behavior (%) Median (Min–Max)[b]	Latency Time of Mosquitoes Until Landing (Min) Median (Min–Max)[c]
a (2 M)	30	357 (337–378)	49 (36–63)	75 (50–100)	25 (5–53)
b (1 M, 1 F)	46	240 (223–378)	50 (44–55)	55 (50–60)	20 (1–59)
c (2 M)	40	219.3 (217–221)	60 (47–64)	18 (18–18)	31 (1–59)
f (2 M, 4 F)	170	146 (9–370)	51 (35–72)	40 (12–80)	25 (1–60)
g (1 M, 3 F)	98	463 (329–1387)	38 (30–52)	32 (0–33)	23 (3–59)
i (5 M, 4 F)	260	115 (82–234)	53 (34–73)	20 (0–35)	25 (1–59)

[a] Kruskal–Wallis–ANOVA: H (5, 25)=4.3; P=0.5
[b] Kruskal–Wallis–ANOVA: H (5, 25)=11.13; P=0.05
[c] ANOVA: F (5; 314) = 0.33; P=0.89

methods, but probably such differences are also due to sampling of plants of different geographical origin. Different populations of *S. otites* emit population-specific scent profiles with only 19 out of 38 inflorescence volatiles being common to plants of the 9 populations studied here.

Although intraspecific variation in floral scent has been observed for many angiosperms, comprehensive screening for population/geographic variation in floral scent composition has been investigated only in few species, e.g., *Yucca filamentosa* L (Agavaceae; Svensson et al. 2005), *Magnolia kobus* DC (Magnoliaceae; Azuma et al. 2001), *Geonoma macrostachys* Mart. (Arecaceae; Knudsen 2002), *Silene latifolia* L. (Caryophyllaceae; Dötterl et al. 2005b), and *Ophrys* species (Orchidaceae; Mant et al. 2005). The intraspecific variability found in our dataset was comparable to variability found in other studied taxa. Such variability may be the result of genetic drift or natural selection (Tollsten and Bergström 1993). Furthermore, different chemotypes may be adapted to different pollinators (Whitten and Williams 1992; Tollsten and Bergström 1993).

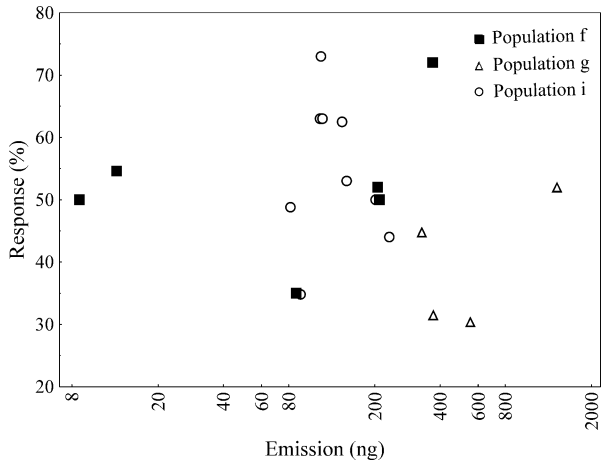

Fig. 3 Total amount of scent emission from *S. otites* inflorescences and % mosquitoes attracted in the wind tunnel (N=100%=170, 98, 260 for 'f', 'g', and 'i' population, respectively)

So far, we do not know the evolutionary factors that trigger the observed odor variability among *S. otites* populations. Different pollinators associated with the different populations might exert different selective pressures on the odor. Only a few species of nocturnal Lepidoptera and mosquitoes have been recorded as pollinators in this species (Brantjes and Leemans 1976); among them, *Autographa gamma* L. and *Culex pipiens*. Whereas *A. gamma* is known to be strongly attracted by lilac aldehyde (Plepys et al. 2002a, b), *C. pipiens* is known to respond strongly to phenyl acetaldehyde (Jhumur et al. 2006).

In this study, phenyl acetaldehyde was the dominant and abundant odor compound, followed by lilac aldehyde, (*Z*)-3-hexenyl acetate, linalool oxide (pyranoid), (*Z*)-3-hexen-1-ol, benzaldehyde, phenylethyl alcohol, linalool, linalool oxide (furanoid), lilac alcohol, acetophenone, methyl salicylate, and hexanol. Most of these compounds are known to elicit strong antennal responses and/or to be attractive to moths such as *Hadena bicruris* Hufn. (Lepidoptera: Noctuidae, Dötterl et al. 2006b), *Sphinx perelegans* Edwards (Lepidoptera: Sphingidae, Raguso and Light 1998), *Hyles lineata* L. (Lepidoptera: Sphingidae, Raguso et al. 1996), *Argyresthia conjugella* Zeller (Lepidoptera: Argyresthiidae, Bengtsson et al. 2007), *Cydia pomenella* L. (Lepidoptera: Tortricidae, Bengtsson et al. 2007), and *Mamestra brassicae* L. (Lepidoptera: Noctuidae, Rojas 1999), whereas only phenyl acetaldehyde has been reported as being attractive to mosquitoes (Howse 2003; Jhumur et al. 2006). It is interesting to note that 19 out of 35 identified compounds in *S. otites* were also found in other closely related *Silene* species, which have been described as moth-pollinated flowers (Jürgens et al. 2002). Thus, it is not surprising that besides mosquitoes, moths

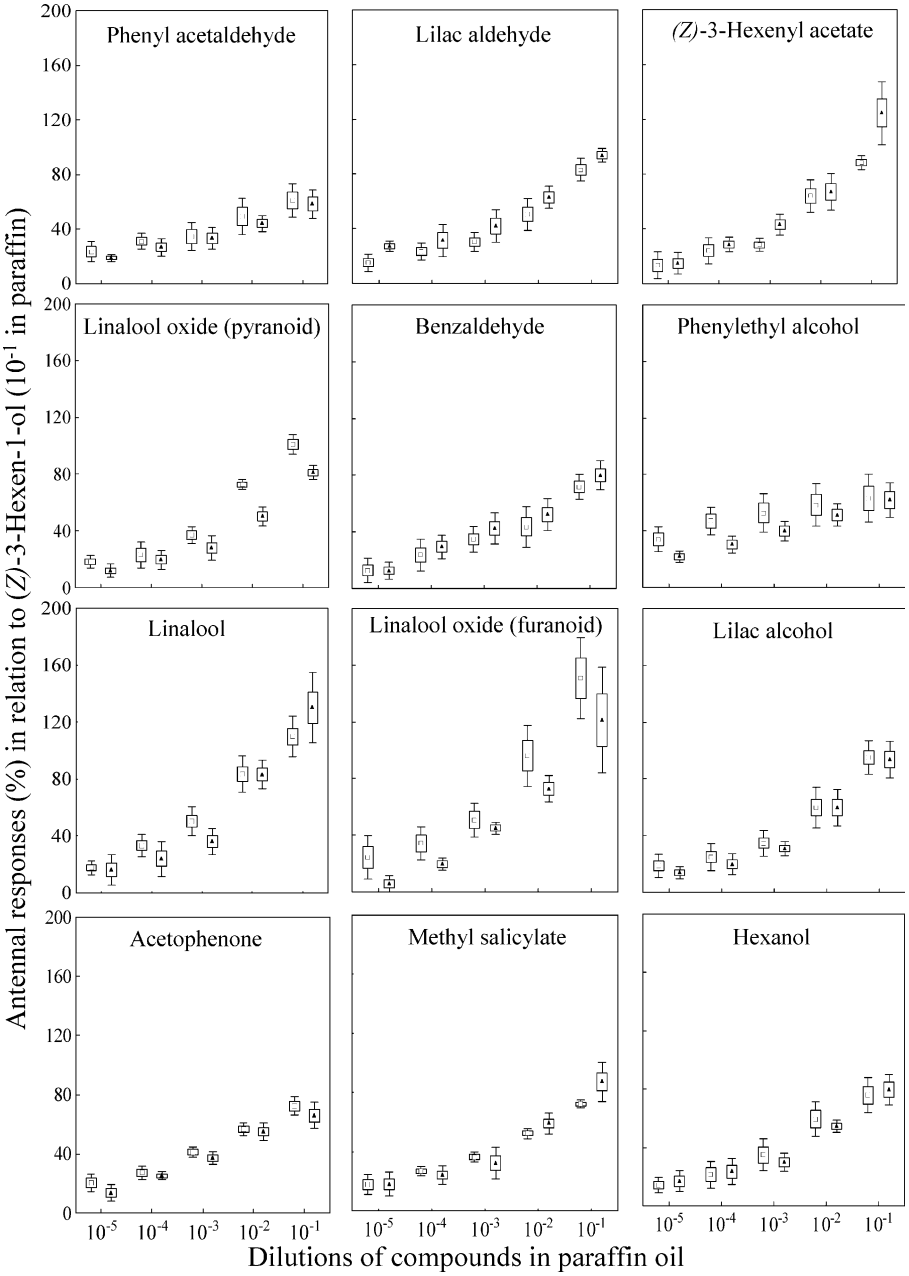

Fig. 4 EAG responses of male (*rectangular*) and female (*triangular*) *Culex pipiens pipiens* biotype *molestus* to different dilutions (in paraffin) of common floral scent compounds of *S. otites* of different populations. Twenty microliters of each dilution of 12 scent compounds were tested on 4–6 mosquitoes. The antennal responses are given in relation to a standard stimulus (Z-3-Hexen-1-ol). Odor compounds have been sorted according to their mean percentage amounts in *S. otites*. All monoterpenoids were used as stereoisomeric mixtures

have also been reported as pollinators of *S. otites* (Brantjes and Leemans 1976).

Our study showed that in the absence of visual stimuli, mosquitoes were attracted to male and female inflorescences of *S. otites* by scent only. The attractiveness of both sexes of this dioecious plant was similarly strong in bioassays, although female and male inflorescences differed with respect to the relative amounts of scent compounds. We found no significant differences in intensity or latency time of response to the inflorescence scents of six different populations. Therefore, different compound mixtures seem to have the same attractiveness.

Table 3 Multiple comparisons based on a GLM of antennal responses of male and female mosquitoes to different compounds and dilutions

Effect	*df*	MS	*F*	*P*
Intercept	1	1,181,681	11,128.31	<0.001
Sex	1	569	5.36	0.02
Dilution	4	92,933	875.18	<0.001
Compound	12	2,603	24.51	<0.001
Sex × dilution	4	87	0.82	0.511
Sex × compound	12	841	7.92	<0.001
Dilution × compound	48	960	9.05	<0.001
Sex × dilution × compound	48	135	1.27	0.116
Error	455	106		

Even within *S. otites* populations that showed low qualitative and semiquantitative scent variation, no positive relation between the total amount of scent emitted and the number of mosquitoes attracted was found. This finding is in contrast to the results of Bowen (1992) who found that behavioral response increased with stimulus concentration. Microclimatic conditions in the wind tunnel, such as temperature (which ranged from 20°C to 25°C), humidity, and atmospheric pressure, might have influenced the results obtained in this study (Grimstad and DeFoliart 1975). Furthermore, inflorescences might have emitted not only attractive compounds, but also compounds repellent to mosquitoes (Kessler and Baldwin 2007). The effect of repellency could increase with increasing concentration of these repellent compounds. Jhumur et al. (2006) found that the dominant odor compound of *S. otites*, phenyl acetaldehyde, attracted about 65% of *C. p. molestus*, whereas only about 50% of the mosquitoes were attracted to the entire *S. otites* inflorescence odor in our study. This finding supports the hypothesis that *S. otites* emits not only attractive, but also repellent compounds.

EAG studies were conducted to examine whether mosquitoes are able to detect components of the *S. otites* odor profile other than phenyl acetaldehyde. Mosquitoes responded to all tested compounds, and all may be involved in host-plant finding by mosquitoes. Bioassays are needed to test the behavioral response of *C. pipiens* to these compounds. Compounds tested in the EAG studies were representative of the floral scent composition of *S. otites*, accounting for 97% on average of all samples of this species. Both male and female mosquitoes detected all 13 floral scent compounds (including (*Z*)-3-hexen-1-ol) till 10^{-5} dilutions. Therefore, if some of these compounds also prove to be attractive, then they might be used as reliable cues for finding *S. otites*, and as long-range attractants by *C. pipiens*.

From this and previous studies, mosquitoes have been proven to detect or be attracted to 15 floral volatiles (Mauer and Rowley 1999; Howse 2003; Kline et al. 2003; Jhumur et al. 2006). It is interesting to note that the ranking of the EAG responses does not correlate with the dominance of the volatiles in floral scent profiles. For example, phenyl acetaldehyde elicited only weak responses in EAGs although it is the main compound (35% mean percentage amount) in the scent of *S. otites*. Furthermore, this compound was attractive to mosquitoes (Jhumur et al. 2006). On the other hand, the mean percentage amounts of linalool and linalool oxide (furanoid) were only 3% and 2%, respectively, but elicited the strongest EAG responses.

Several studies provide evidence that release of linalool oxide (furanoid) and linalool may reflect adaptations by plants to attract lepidopteran pollinators (Raguso et al. 1996; Raguso and Light 1998; Andersson et al. 2002; Andersson and Dobson 2003). Linalool also occurs in plants pollinated by bats, bees, flies, beetles, and wasps (Borg-Karlson et al. 1996; Raguso and Pichersky 1999). These monoterpenoids may also be important for attraction of mosquitoes and could explain the mixed pollinator guild found in *S. otites*, mainly moths and mosquitoes. Indeed, the attractiveness of linalool for mosquitoes was confirmed by Kline et al. (2003). In a dual-port olfactometer, more *Aedes aegypti* (L.) individuals were attracted by linalool than by a control. Although these two oxygenated monoterpenes are generally assumed to be pollinator attractants, Ômura et al. (2000) reported that linalool oxide (furanoid) acted as a weak deterrent in proboscis extension responses and a weak repellent in flower alighting tests with the cabbage butterfly *Pieris rapae* L., indicating that this compound can be repellent and attractive to insects. Bioassays are needed to determine the behavioral response to linalool oxide in mosquitoes.

Similarly to phenyl acetaldehyde, phenylethyl alcohol (3% mean percentage) elicited only weak EAG responses, although this compound may also be attractive to mosquitoes. Mauer and Rowley (1999) found that *C. pipiens* was attracted to the scent of the common milkweed *Asclepias syriaca* L., which is dominated by phenylethyl alcohol and benzyl alcohol. The authors assumed that these two benzenoids were responsible for the attraction of mosquitoes to *A. syriaca*, but they failed to attract mosquitoes in a dual-port olfactometer to a synthetic mixture of these two compounds.

It is interesting to note that in our study, *C. pipiens* also responded to the typical green leaf odors of *S. otites*, such as (*Z*)-3-hexenyl acetate. These compounds are not only released from several plant species in response to herbivory, but also serve as attractants for a variety of predatory and parasitic insects (see Röse et al. 1998; James 2005). In the natural environment, green leaf compounds are widespread and would not necessarily guide insects directly to flowers (Honda et al. 1998), although being directed to vegetation would certainly increase the probability of finding flowers.

In summary, floral scent compositions of *S. otites* populations from different geographical origin are highly variable, but nevertheless similarly attractive to *Culex pipiens molestus* mosquitoes. Mosquitoes can detect the most common and abundant scent compounds of *S. otites* inflorescences, but knowledge of the biological significance of most of the compounds is still lacking (e.g., attractant or repellent). By means of bioassays, we are presently evaluating the role of these compounds in the plant–pollinator interactions of *S. otites* and mosquitoes, which might lead to the development of new means of pest control and mosquito attractants and repellents.

Acknowledgements The authors thank Sigrid Liede-Schumann for supporting this study and Taina Witt who gave valuable comments on earlier versions of the manuscript. Karlheinz Seifert and Jette Knudsen provided authentic standard compounds. Rainer Krug and Deinlein Heike helped in the cultivation of the plants. The comments of Monika Hilker and two anonymous referees were especially helpful for the improvement of the manuscript. Umma Salma Jhumur was funded by the German Research Foundation (DFG Research Training Group 678).

References

ADAMS, R. P. 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publishing Corporation, Carol Stream, Illinois.

ANDERSSON, S., and DOBSON, H. E. M. 2003. Antennal responses to floral scents in the butterfly *Heliconius melpomene*. *J. Chem. Ecol* 29:2319–2330.

ANDERSSON, S., NILSSON, L. A., GROTH, I., and BERGSTRÖM, G. 2002. Floral scents in butterfly-pollinated plants: possible convergence in chemical composition. *Bot. J. Linn. Soc* 140:129–153.

AYASSE, M., SCHIESTL, F. P., Paulus, H. F., LÖFSTEDT, C., HANSSON, B., IBARRA, F., and FRANCKE, W. 2000. Evolution of reproductive strategies in the sexually deceptive orchid *Ophrys sphegodes*: How does flower-specific variation of odor signals influence reproductive success. *Evolution* 54:1995–2006.

AZUMA, H., TOYOTA, M., and ASAKAWA, Y. 2001. Intraspecific variation of floral scent chemistry in *Magnolia kobus* DC. (Magnoliaceae). *J. Plant Res* 114:411–422.

BENGTSSON, M. C., WITZGALL, P., KOBRO, S., JAASTAD, G., LOFQVIST, J. and LINDHE, C. 2007. Attractant for apple fruit moth and other insect pests of apple. United States Patent: 20070004686 A1.

BORG-KARLSON, A. K., UNELIUS, C. R., VALTEROVÁ, I., and NILSSON, L. A. 1996. Floral fragrance chemistry in the early flowering shrub *Daphne mezereum*. *Phytochemistry* 41:1477–1483.

BOWEN, M. F. 1992. Terpene sensitive receptors in female *Culex pipiens* mosquitoes: electrophysiology and behavior. *J. Insect Physiol* 38:759–764.

BRANTJES, N. B. M., and LEEMANS, J. A. A. M. 1976. *Silene otites* (Caryophyllaceae) pollinated by nocturnal Lepidoptera and mosquitoes. *Acta Bot. Neerl* 25:281–295.

CLARKE, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol* 18:117–143.

CLARKE, K. R., and WARWICK, R. M. 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd edition. Primer-E, Plymouth.

CLARKE, K. R. and GORLEY, R. N. 2006. Primer v6: User Manual/Tutorial. Primer-E, Plymouth.1–91.

DÖTTERL, S., FÜSSEL, U., JÜRGENS, A., and AAS, G. 2005a. 1,4-Dimethoxybenzene, a floral scent compound in willows that attracts an oligolectic bee. *J. Chem. Ecol* 31:2993–2998.

DÖTTERL, S., WOLFE, L. M., and JÜRGENS, A. 2005b. Qualitative and quantitative analyses of flower scent in *Silene latifolia*. *Phytochemistry* 66:203–213.

DÖTTERL, S., BURKHARDT, D., WEIßBECKER, B., JÜRGENS, A., SCHÜTZ, S., and MOSANDL, A. 2006a. Linalool and lilac aldehyde/alcohol in flower scents. Electrophysiological detection of lilac aldehyde stereoisomers by a moth. *J. Chromatograph. A* 1113:231–238.

DÖTTERL, S., JÜRGENS, A., SEIFERT, K., LAUBE, T., WEIßBECKER, B., and SCHÜTZ, S. 2006b. Nursery pollination by a moth in *Silene latifolia*: the role of odours in eliciting antennal and behavioural responses. *New Phytol* 169:707–718.

DUDAREVA, N., and PICHERSKY, E. 2000. Biochemical and molecular genetic aspects of floral scents. *Plant Physiol* 122:627–633.

GADAWASKI, R., and SMITH, S. M. 1992. Nectar sources and age structure in a population of *Aedes provocans* (Diptera: Culicidae). *Entomol. Soc. Am* 29:876–886.

GRIMSTAD, P. R., and DEFOLIART, G. R. 1974. Nectar sources of Wisconsin mosquitoes. *J. Med. Entomol* 11:331–341.

GRIMSTAD, P. R., and DEFOLIART, G. R. 1975. Mosquito nectar feeding in Wisconsin in relation to twilight and microclimate. *J. Med. Entomol* 11:691–698.

HAEGER, J. S. 1955. The non-blood feeding habits of *Aedes taeniorhyndus* (Diptera, Culicidae) on Sanibel Island, Florida. *Mosq. News* 15:21–26.

HARADA, F., MORIYA, K., and YABE, T. 1971. Observations on the survival and longevity of the adult *Culex* mosquitoes fed on the flowers of some nectar plants. *Jpn. J. Sanit. Zool* 22:18–23.

HONDA, K., ÔMURA, H., and HAYASHI, N. 1998. Identification of floral volatiles from *Ligustrum japonicum* that stimulate flower visiting by cabbage butterfly, *Pieris rapae*. *J. Chem. Ecol* 24:2167–2180.

HOWSE, E. P. 2003. Insect attractant. European Patent Specification EP 0 838998 B1.

JAMES, D. G. 2005. Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *J. Chem. Ecol* 31:481–495.

JHUMUR, U. S., DÖTTERL, S., and JÜRGENS, A. 2006. Naïve and conditioned responses of *Culex pipiens pipiens* biotype *molestus* (Diptera: Culicidae) to flower odors. *J. Med. Entomol* 43:1164–1170.

JÜRGENS, A., WITT, T., and GOTTSBERGER, G. 2002. Flower scent composition in night-flowering *Silene* species (Caryophyllaceae). *Biochem. Syst. Ecol* 30:383–397.

KESSLER, D., and BALDWIN, I. T. 2007. Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*. *Plant J* 49:840–854.

KLINE, D. L., BERNIER, U. R., POSEY, K. H., and BARNARD, D. R. 2003. Olfactometric evaluation of spatial repellents for Aedes aegypti. *J. Med. Entomol* 40:463–467.

KLOWDEN, M. J. 1986. Effects of sugar deprivation on the host-seeking behavior of gravid Aedes aegypti mosquitoes. *J. Insect Physiol* 32:479–483.

KNUDSEN, J. T. 2002. Variation in floral scent composition within and between populations of *Geonoma macrostachys* (Arecaceae) in the western Amazon. *Am. J. Bot* 89:1772–1778.

KNUDSEN, J. T., ERIKSSON, R., GERSHENZON, J., and STÅHL, B. 2006. Diversity and distribution of floral scent. *Bot. Rev* 72:1–120.

MAGNARELLI, L. A. 1978. Nectar-feeding by female mosquitoes and its relation to follicular development and parity. *J. Med. Entomol* 14:527–530.

MANT, J., PEAKALL, R., and SCHIESTL, F. P. 2005. Does selection on floral odor promote differentiation among populations and species of the sexually deceptive orchid genus Ophrys. *Evolution* 59:1449–1463.

MAUER, D. J., and ROWLEY, W. A. 1999. Attraction of *Culex pipiens pipiens* (Diptera: Culicidae) to flower volatiles. *J. Med. Entomol* 36:503–507.

NAYAR, J. K., and SAURMAN, D. M. 1971. The effect of diet on life-span, fecundity and flight potential of female *Aedes taeniorhynchus* adults. *J. Med. Entomol* 8:506–513.

NAYAR, J. K., and SAURMAN, D. M. 1975. The effects of nutrition on survival and fecundity in Florida mosquitoes. Part 1. Utilization of sugar for survival. *J. Med. Entomol* 12:92–98.

ÔMURA, H., HONDA, K., and HAYASHI, N. 2000. Floral scent of *Osmanthus fragrans* discourages foraging behavior of cabbage butterfly, *Pieris rapae*. *J. Chem. Ecol* 26:655–666.

PLEPYS, D., IBARRA, F., FRANCKE, W., and LÖFSTEDT, C. 2002a. Odour-mediated nectar foraging in the silver Y moth, *Autographa gamma* (Lepidoptera: Noctuidae): behavioural and electrophysiological responses to floral volatiles. *Oikos* 99:75–82.

PLEPYS, D., IBARRA, F., and LÖFSTEDT, C. 2002b. Volatiles from flowers of *Platanthera bifolia* (Orchidaceae) attractive to the silver Y moth, *Autographa gamma* (Lepidoptera: Noctuidae). *Oikos* 99:69–74.

RAGUSO, R. A., and LIGHT, D. M. 1998. Electroantennogram responses of male *Sphinx perelegans* hawkmoths to floral and 'green-leaf volatiles. *Entomol. Exp. Appl* 86:287–293.

RAGUSO, R. A., and PICHERSKY, E. 1999. A day in the life of a linalool molecule: Chemical communication in a plant-pollinator system. Part 1: Linalool biosynthesis in flowering plants. *Plant Species Biol* 14:95–120.

RAGUSO, R. A., LIGHT, D. M., and PICKERSKY, E. 1996. Electroantennogram responses of *Hyles lineata* (Sphingidae: Lepidoptera) to volatile compounds from *Clarkia breweri* (Onagraceae) and other moth-pollinated flowers. *J. Chem. Ecol* 22:1735–1766.

ROJAS, J. C. 1999. Electrophysiological and behavioural responses of the cabbage moth to plant volatiles. *J. Chem. Ecol* 25:1867–1883.

RÖSE, U. S. R., LEWIS, W. J., and TUMLINSON, J. H. 1998. Specificity of systemically released cotton volatiles as attractants for specialist and generalist parasitic wasps. *J. Chem. Ecol* 24:303–319.

SANDHOLM, H. A., and PRICE, R. D. 1962. Field observations on the nectar feeding habits of some Minnesota mosquitoes. *Mosq. News* 22:346–349.

SCHÜTZ, S., WEIßBECKER, B., KOCH, U. T., and HUMMEL, H. E. 1999. Detection of volatiles released by diseased potato tubers using a biosensor on the basis of intact insect antennae. *Biosens. Bioelectron* 14:221–228.

Statsoft, Inc. 2004. STATISTICA for Windows Version 7. www.statsoft.com.

STOUTAMIRE, W. P. 1968. Mosquito pollination of *Habenaria obtusata* (Orchidaceae). *Mich. Bot* 7:203–212.

SVENSSON, G. P., HICKMAN, M. O., BARTRAM, S., BOLAND, W., PELLMYR, O., and RAGUSO, R. A. 2005. Chemistry and geographic variation of floral scent in *Yucca filamentosa* (Agavaceae). *Am. J. Bot* 92:1624–1631.

TOLLSTEN, L., and BERGSTRÖM, G. 1993. Fragrance chemotypes of *Platanthera* (Orchidaceae): The result of adaptation to pollinating moths. *Nord. J. Bot* 13:607–613.

VARGO, A. M., and FOSTER, W. A. 1982. Responsiveness of female *Aedes aegypti* (Diptera, Culicidae) to flower extracts. *J. Med. Entomol* 19:710–718.

WHITTEN, W. M., and WILLIAMS, N. H. 1992. Floral fragrances of *Stanhopea* (Orchidaceae). *Lindleyana* 7:130–153.