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Volatile Organic Compounds as Signals in a Plant–Herbivore System: Electrophysiological Responses in Olfactory Sensilla of the Moth *Cactoblastis cactorum*

Blanka Pophof[1,2], Gert Stange[3] and Leif Abrell[4,5]

[1]Max-Planck-Institut for Behavioral Physiology, Seewiesen, Germany, [3]Research School of Biological Sciences, Australian National University, Canberra ACT 0200, Australia and [4]Biosphere 2 Chemistry Unit, Columbia University, Oracle, AZ 85623, USA
[2]Present address: Federal Office for Radiation Protection, D-85764 Oberschleissheim/ Neuherberg, Germany
[5]Present address: Department of Chemistry, University of Arizona, Tucson, AZ 85721, USA

Correspondence to be sent to: PD Dr. Blanka Pophof, Bundesamt für Strahlenschutz, Ingolstädter Landstrasse 1, D-85764 Oberschleissheim/ Neuherberg, Germany. E-mail: bpophof@bfs.de

Abstract

The morphological sensillum types on the antennae of male and female *Cactoblastis cactorum* were visualized by scanning electron microscopy. Electrophysiological recordings were performed for the first time on single olfactory sensilla of *C. cactorum*. The male sensilla trichodea house a receptor cell responding to the putative pheromone component (9*Z*,12*E*)-tetradecadienyl acetate. The sensilla trichodea of the females were much shorter than those of the males and contained specialized receptor cells responding to certain terpenoids, the most frequent being the nerolidol-sensitive cell. The sensilla auricillica and sensilla basiconica of both sexes contained cells responding less specifically to terpenoid compounds as well as to green leaf volatiles. Cells of the sensilla coeloconica responded to aliphatic aldehydes and acids. Eight volatile organic compounds emitted by *Opuntia stricta*, a host plant of *C. cactorum*, were identified using gas chromatography–mass spectrometry, β-caryophyllene being the major compound. Five compounds identified by gas chromatography in the headspace of *O. stricta* elicited responses in olfactory receptor cells of *C. cactorum*, nonanal being the most active compound and therefore a candidate attractant of *C. cactorum*.

Key words: *Cactoblastis cactorum*, gas chromatography–mass spectrometry, *Opuntia stricta*, pheromones, single sensillum recordings, volatile organic compounds

Introduction

The interaction between phytophagous insects and their hosts is partially mediated by volatile organic compounds (VOCs) that are synthesized as products of plant metabolism and emitted into the environment. Within the context of moth olfactory orientation, VOCs often form sensory cues (e.g. Todd and Baker, 1993; Anderson *et al*., 1995; Røstelien *et al*., 2000a,b; Stranden *et al*., 2002) that define host specificity and may provide information for behavioral choice of feeding and oviposition sites (Müller and Hilker, 2000, 2001; for a review see Honda, 1995). In addition, VOCs may act as cues for the selection of a particular individual of a given host species or as deterrents if they are specifically emitted by a damaged individual (De Morales *et al*., 2001). Volatiles induced by the attack of pest insects may even attract their natural enemies to the infested plants (e.g. Hilker *et al*., 2002; for reviews on induced volatiles, see Dicke and van Loon, 2000; Dicke and Hilker, 2003).

An examination of the role of VOCs in the interaction between the moth *Cactoblastis cactorum* and its cactus hosts of the genus *Opuntia* is interesting from several perspectives. First, *C. cactorum* has been a classic example of the successful biological control of a particularly catastrophic weed infestation: by 1925 an introduced plant, the prickly pear cactus *Opuntia stricta* had restricted human access to 25 million hectares of eastern Australia and was continuing its advance at ~100 hectares/h (Osmond and Monro, 1981). In 1925, after several unsuccessful attempts of chemical and biological control by other methods, *C. cactorum*, a native of Argentina and Paraguay, was introduced to Australia and proved to be a successful control agent by near-complete destruction of the cactus population. Eighty years later, the prickly pear and the moth continue to coexist, albeit at much lower and sustainable population densities.

Secondly and paradoxically, the biological control agent now shows potential as an insect pest to threaten a major native ecosystem in a different part of the world. *Opuntia stricta* cacti which became known as pest pears in Australia are native to Louisiana and its adjacent desert states in the USA. Until recently, the geographical separation between the original South American habitat of *C. cactorum* and the cactus habitats in North America was apparently sufficient to prevent infestation, but this has now been breached by human interference. In 1957, egg sticks were sent to Nevis in the Caribbean, in order to control local *Opuntia* spp. (Simmonds and Bennett, 1966). From Nevis, *C. cactorum* spread to other Caribbean islands, partially by deliberate introduction and partially by natural migration (Garcia-Tuduri *et al*., 1971; Bennett *et al*., 1985; Starmer *et al*., 1987). In 1989, the moth was discovered on the North American mainland in Florida. *Cactoblastis cactorum* is now established there, with the result that indigenous cacti such as the semaphore cactus, *O. spinosissima*, have become critically endangered (Zimmermann *et al*., 2001). A further spread of the moth towards the US desert states and Mexico is possible, adding relevance to research into mechanisms of host selection.

Finally, the *Cactoblastis–Opuntia* system is of particular theoretical interest because the host uses the crassulacean acid metabolism pathway (CAM, Osmond *et al*., 1979; Black and Osmond, 2003) for CO_2 fixation. Although CAM includes all enzymes required for both C_3 and C_4 metabolism, resulting metabolic by-products may differ between CAM and C_3 or C_4 plants. CAM volatiles reported to date from non-cacti (e.g. in the tree *Clusia rosea* and pineapple; see Lerdau and Keller, 1997; Nogueira *et al*., 2001; Preston *et al*., 2003) do not notably differ from C_3 or C_4 plant VOCs. An objective of the present paper is the presentation of a survey of VOCs from the headspace of *O. stricta*.

From a chemoecological perspective, it is interesting that CAM metabolism allows the temporal separation between photosynthesis and CO_2 fixation, meaning that the plants' stomata are often open in darkness, assimilating CO_2 and releasing VOCs. *Cactoblastis cactorum* are nocturnally active moths. In previous work, it has been shown that the latter aspect is relevant for host finding by *Cactoblastis*: the CO_2 gradient on the surface of assimilating cladodes of *O. stricta* forms a close-range sensory cue for oviposition (Stange *et al*., 1995), and artificially induced fluctuations of CO_2 concentration deter oviposition (Stange, 1997). CO_2, as a major atmospheric trace gas, is not a VOC in the strict sense and the constraints on its sensory perception are different from those applying to VOCs. In particular, the ubiquitous occurrence of CO_2 sources or sinks makes it unlikely that CO_2 gradients alone are sufficient sensory cues to exclusively define host specificity, and the mechanisms of atmospheric dispersal of CO_2 gradients suggest that they are not important for long distance orientation. Therefore, it is desirable to supplement existing findings by addressing the role of more specific VOCs, and one objective of the present work is to obtain data on both the spectrum of VOCs emitted by the plant host and the spectrum of sensitivities found in the antennal sensory receptors of the moth.

Currently, no information exists about VOCs from *O. stricta* that may serve as olfactory cues for *C. cactorum* host plant finding. In fact, relatively little is known about cactus volatile chemistry. VOCs reported from cacti so far mostly include floral volatiles (Kaiser and Tollsten, 1995), such as the novel dehydrogeosmin and other terpenoids, benzyl derivatives, sulfur-containing compounds attractive to pollinating bats and lipoxogenase products, all from non-platyopuntias (Kaiser and Nussbaumer, 1990; Kaiser and Tollsten, 1995). In order to find chemical cues relevant for *C. cactorum* to locate its host plant, solid-phase microextraction (SPME) (Pawliszyn, 1999) was used to collect VOCs from *O. stricta* non-flowering cladodes followed by gas chromatography–mass spectrometry (GC-MS) to analyze these VOCs after desorption from the SPME fibers. As a prerequisite for electrophysiological recording, the morphology and distribution of the various sensillum types on the antennae of male and female *C. cactorum* was studied by scanning electron microscopy. Finally, the responses of these sensilla to identified and putative VOCs of *O. stricta* were characterized electrophysiologically by means of electroantennography and single sensillum recording techniques.

Materials and methods

Animals

Cactoblastis-infested cladodes of *O. stricta* were collected in Araluen, New South Wales, Australia and kept in a greenhouse until pupation had occurred. For electrophysiological experiments, the pupae were shipped to Germany, sexed, and then kept at room temperature (20°C) and 16:8 light/dark regime until emergence. Adults were kept in Petri dishes at 7°C and high humidity (supplied by a water soaked cotton pad) until used for experiments. In total, 24 males (0–11 days after emergence) and 13 females (1–5 days after emergence) were used for electrophysiological recordings.

Morphology

Antennae of male and female *C. cactorum* were studied after standard preparation (air drying, gold sputtering; see Hallberg *et al*., 1994) in a Hitachi 4500 field emission scanning electron microscope.

VOC analysis by GC-MS

Solid phase microextraction fibers (Supelco, Bellefonte, PA; Pawliszyn, 1999) were used to collect VOCs from *O. stricta* followed by desorption into the GC for GC-MS analysis. Thirteen potted *O. stricta* plants were purchased from Hoak's Greenhouse & Nursery, Inc. Homestead, FL, and grown inside a greenhouse (~25°C daytime temperature, natural light

and day-length conditions near Oracle, Arizona, USA (32°35′N, 110°51′W, 1165 m elevation) for 10 weeks. One plant was removed from its 15 cm diam. pot and the soil was shaken from the roots and collected. The soil was returned to the same pot without the cactus plant to serve as a negative control for VOC analysis. Polyvinylacetate bags (Reynolds Inc., 1 l volume) (Raguso and Pellmyr, 1998) were placed around the soil-only pot, and around a single non-flowering *O. stricta* plant with a wire frame attached to the pot extending above the plant to prevent the bag from touching the plant. The soil-only pot was sampled after 17 h of enclosure in the bag (overnight) by piercing the bag with the SPME needle and extending a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber into the bag for 10 min to allow absorption of VOCs. The pot with *O. stricta* was sampled five times after 70 h (three overnights) in the same way with the SPME needle and PDMS/DVB fiber for 10 min. Fiber desorption was conducted for 5 min for each sample into the injection port of a Hewlett Packard 5890 GC using splitless injection and a Supelco Equity-1 column (100% PDMS; 30 m × 0.25 μm × 0.25 mm) and the following temperature program: 2 min at 45°C, increasing at 30°C/min up to 120°C; 5 min at 120°C, increasing at 30°C/min up to 220°C; finishing at 220°C for 10 min. VOCs eluting from the GC column were observed as a total ion current (TIC) chromatogram using Chemstation software and a Hewlett Packard 5971 mass selective detector (ionization energy 70 eV). Retention times of the VOCs found in *O. stricta* and seven commercially available standards (Sigma-Aldrich: α-pinene, β-pinene, nonanal, copaene, β-caryophyllene, α-caryophyllene; Treat USA, Lakeland, FL: germacrene D (40% minimum) from ylang-ylang oil) were compared.

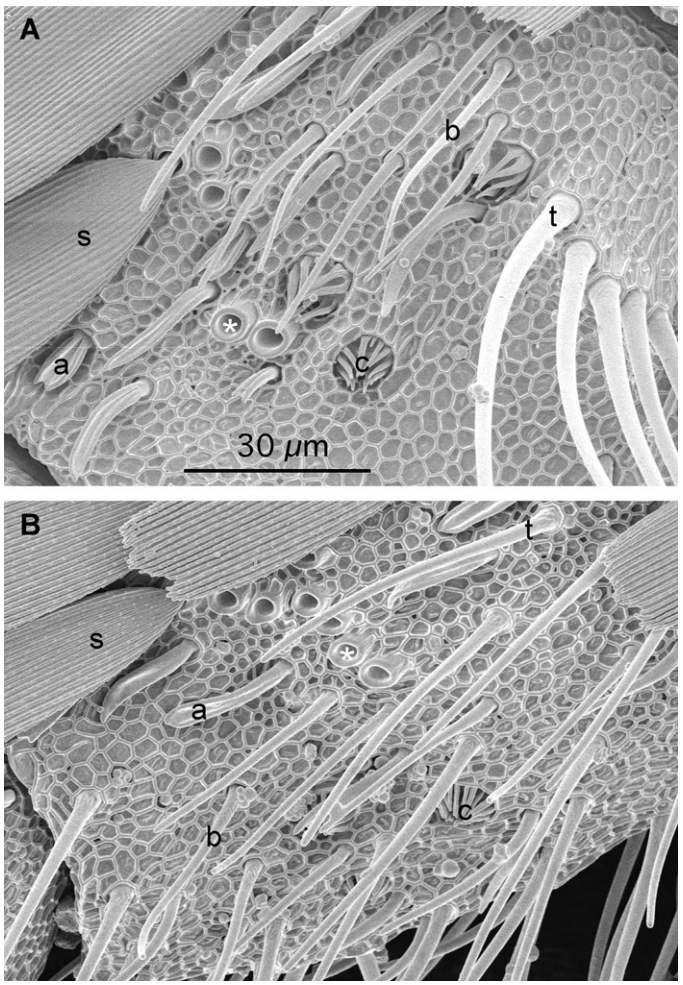

Figure 1 Scanning electron micrographs of a male **(A)** and female **(B)** antenna of *Cactoblastis cactorum*. a, s. auricillicum; b, s. basiconicum; c, s. coeloconicum; s, scale (on dorsal part of antenna); t, s. trichodeum. Asterisk denotes base of removed scale.

Electrophysiological recordings

Electrophysiological recordings were performed using glass capillary Ag–AgCl electrodes. For electroantennographic (EAG) recordings, one antenna was cut off and slipped over the reference electrode; the recording electrode was inserted into the cut tip of the antenna. In this case, both electrodes had a tip diameter of 10 μm and were filled with hemolymph Ringer solution (Kaissling, 1995).

Single sensillum recordings were performed on living moths mounted in plastic pipette tips with antennae fixed by dental wax. The tip of one antenna was cut off and the reference electrode with a tip diameter of 10 μm, filled with hemolymph Ringer solution, was inserted into the open end. The recording electrodes were filled with sensillum–lymph Ringer solution (Kaissling, 1995) and positioned under a stereo microscope (Leitz, magnification ×192). Recording electrodes with a tip diameter of 10 μm were slipped over the tips of single sensilla (s.) trichodea which had been cut using modified forceps (Kaissling, 1995). Electrodes with tip diameter of 1 μm were inserted at the base of s. auricillica or s. basiconica or into the cavities of s. coeloconica (Pophof, 1997).

Table 1 Characteristic properties of VOCs identified in the headspace of *O. stricta* by solid phase microextraction (SPME)

Compound	Retention time (min)	Mol. wt	Confidence level (%)	% of TIC
α-Pinene	5.7	136	91	3.9
β-Pinene	6.1	136	90	0.5
Meta-cymene[a]	6.6	134	64	6.9
Nonanal	7.5	142	87	2.9
Copaene	12.3	204	94	0.5
β-Caryophyllene	12.8	204	99	7.1
Germacrene D	12.9	204	90	0.4
α-Caryophyllene	13.1	204	98	1.7
2,4-Dimethylundecane	13.7	184	86	0.4

[a]Possibly an artifact of SPME procedure (Zabaras and Wyllie, 2002); TIC, total ion current.

The preparation was held in a continuous air stream (1 m/s) filtered through charcoal and humidified by percolation through distilled water. The odorants were loaded on pieces of filter paper (1 cm^2) placed in glass cartridges (7 mm inner diameter). During stimulation the continuous air stream was passed for 500 ms through the cartridge.

Signals from olfactory sensilla were amplified using a custom-made amplifier with a low pass cut-off frequency of 2 kHz. Unfiltered data were sampled on-line using a Macintosh G3 computer and data acquisition program SuperScope II 2.31 (GW Instruments). Spikes of single receptor cells were discriminated visually according to their amplitude. The following parameters of responses to stimulation by volatiles were measured: amplitude of the electroannogram response, amplitude of the receptor potential recorded from single sensilla and peak spike frequency calculated from six consecutive spikes with the shortest interspike intervals.

Volatiles

In total, 14 pheromone candidates (14-carbon acetates, alcohols and aldehydes; see Table 2), chosen based on pheromone components known from other Phycitinae species (Arn *et al*., 1992), and 62 putative VOCs (Table 4) were tested. Pheromone components (from the stocks of the late E. Priesner, Seewiesen) were tested at a stimulus load of 10 μg (in 10 μl *n*-hexane) on filter paper. In a few cases, stock pheromone solutions of unknown concentrations had to be used (Table 2). The VOCs were purchased from Dragoco, Sigma, Aldrich and Fluka, the purity was in the range of 95–98%. Jasmine extract, synthetic jasmin oil (Dragoco) and orange oil (Treat) were mixtures of several terpenoids, the orange oil containing 40% germacrene D. The VOCs were tested at a dose of 1 μl loaded on filter paper in 10 μl solution (1 μl VOC added to 9 μl paraffin oil). Glass cartridges containing filter papers loaded with volatiles were used for several days. Between the experiments, they were enclosed in plastic vials and stored in a refrigerator at −20°C. For control, cartridges with clean filter paper without any odorant were used.

For volatiles which regularly excited the olfactory receptor cells [linalool, geraniol, nerolidol, limonene, citral, (*E*)-3-hexenol, (*Z*)-3-hexenal, heptanal, octanal, nonanal, nonanoic acid] dilution series were prepared. The maximum dose was either 1 or 5 μl, and dilution steps were a factor of 10. Pheromones were diluted in hexane (Roth), VOCs in paraffin

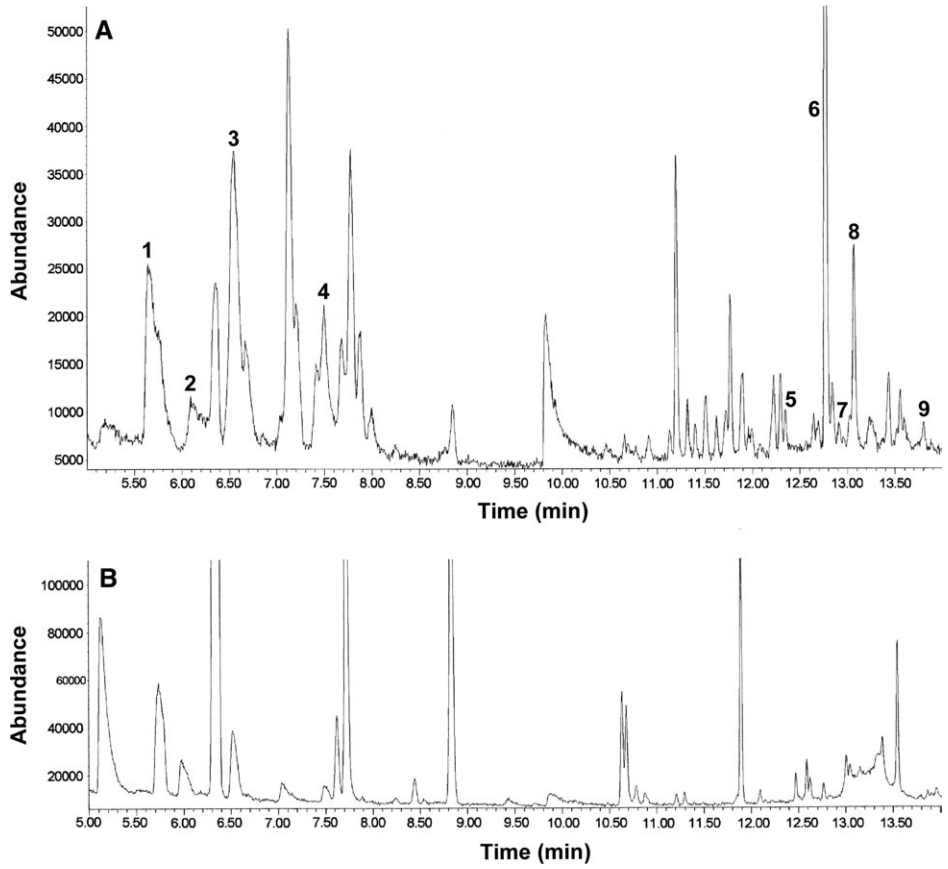

Figure 2 GC-MS total ion current chromatograms from SPME samples of headspace trapped inside polyvinylacetate bags above *Opuntia stricta* **(A)** and a pot with soil only, negative control **(B)**. *Opuntia stricta* VOCs identified include (1) α-pinene, (2) β-pinene, (3) meta-cymene, (4) nonanal, (5) copaene, (6) β-caryophyllene, (7) germacrene D, (8) α-caryophyllene and (9) 2,4-dimethylundecane. Numerous unidentified, branched hydrocarbons were also detected from *O. stricta*. Primary peaks identified from the negative control sample are industrial plastic components and stationary phase silica derivatives.

oil (Roth). To register dose–response dependencies, stimuli of increasing intensity were given at 1 min intervals.

Results

Morphology

The filiform antennae of *C. cactorum* consist of ~100 segments, each carrying a complement of sensilla. As the antennal and sensillar morphology strongly resembles that of other pyralid species (Hallberg *et al*., 1994), the sensillum types were classified accordingly. In the male (Figure 1A), s. trichodea were the most conspicuous, occurring in three regular rows of 6–8 sensilla per segment. In addition, a field of distinct, much smaller, s. basiconica and auricillica was present on each segment, as well as 3–5 s. coeloconica. In the female (Figure 1B), s. auricillica and s. coeloconica were of identical appearance to those in the male but there were no regular rows of s. trichodea. The female s. trichodea were much shorter than in the male; they were straight, but still longer than the slightly bent s. basiconica.

GC-MS

Eight VOCs from *O. stricta* (~24% of all VOCs by peak area) listed in Table 1 were identified with library search confidence levels >85% by matching mass spectra selected from individual total ion current peaks (Figure 2A) with spectra from the Wiley Registry of Mass Spectral Data, 6th Edition (containing 275,000 reference spectra). α- and β-caryophyllenes were detected with the highest confidence of all VOCs (98 and 99%, respectively). β-Caryophyllene was clearly the major VOC detected by solid phase micro-extraction and GC-MS analysis. Meta-cymene (64% confidence level) was possibly an artifact of the SPME procedure (Zabaras and Wyllie, 2002). Retention time comparison between *O. stricta* VOCs and commercially available standards (see Materials and methods) confirmed the identities of the major VOCs shown in Table 1. *Opuntia stricta* VOC identity was also confirmed by comparing mass spectra to spectra obtained from commercially available standards. Numerous unidentified, branched hydrocarbons were also detected from *O. stricta* as other major and minor components (Figure 2A). No *O. stricta* VOCs were detected from the negative control sample (Figure 2B), which provided chromatogram peaks with mass spectra predominantly similar to industrial plastic components and stationary phase silica derivatives.

Physiology: electroantennograms

EAG recordings were performed on one male and three female antennae. In the male antenna, the putative pheromone component (9*Z*,12*E*)-14:Ac elicited a considerable response of 2.2 mV, followed in magnitude by nonanal which evoked a response of 0.9 mV. Other tested compounds—all pheromone candidates and some VOCs—elicited very weak or no

Table 2 Electroantennogram responses to a set of VOCs and putative pheromone components recorded from one male and three female antennae

	Animal			
	M13	F6	F6	F11
Age (day after emergence)	1	0	0	3
Terpenoids				
Monoterpenoids				
Hydrocarbons				
α-Pinene[a]	0	++	0	
α-Terpinene		0	0	
β-Pinene[a]		+	0	
Camphene		0	0	
δ-3-Carene		0	0	
γ-Terpinene		0	0	
Limonene	0	+	0	0
Myrcene		+	+	
Esters				
Citronellyl acetate		+	0	
Linalyl acetate		++	0	
Neryl acetate		++	++	
Terpineol acetate		++	0	
Ketones				
cis-Verbenone		+	0	
Geranyl acetone		+	0	
Neryl acetone	0	+	0	
Thujone		0	0	
Alcohols				
Citronellol		++	+	
Geraniol	0	++	+	+
Linalool	0	++	+	+
Nerol		++	++	
Terpin-4-ol		++	+	
Terpineol	+	++	+	
Thujyl alcohol		0	0	
Aldehydes				
Citral		++	++	+
Sesquiterpenoids				
Hydrocarbons				
α-Caryophyllene[a]		+	0	
β-Caryophyllene[a]		+	0	
Bulnesene		0	0	

Table 2 Continued

	Animal			
	M13	F6	F6	F11
Age (day after emergence)	1	0	0	3
Guajene		0	0	
Murolene		0	0	
Alcohol				
Nerolidol	0	+	+	0
Diterpenes				
Alcohol				
Isophytol		+	0	
Aromatics				
Hydrocarbon				
p-Cymene		+	0	
Ester				
Benzylacetate	0	+++	++	+
Alcohols				
1-Phenylethanol		+	+	
2-Phenylethanol		+	++	
Creosol		+	0	
Eugenol		++	+	
Methyl-eugenol		0	0	
Aldehydes				
Benzaldehyde		+	+	
Phenyl-acetaldehyde	0	++	+	
Carboxylic acid				
Benzoic acid		+		
Fatty acid derivatives				
Esters				
(9*E*,11*E*)-14:Ac	0			
(9*E*,11*Z*)-14:Ac	+			
(9*Z*,11*E*)-14:Ac	0			
(9*Z*,12*E*)-14:Ac[b]	+++	+	+	+
(10*E*,12*Z*)-14:Ac	0			
(10*Z*,12*Z*)-14:Ac	0			
(*E*)-12–14:Ac	0			
(*Z*)-11-Tetradecen-9-yn:Ac	0			
(*Z*)-9–14:Ac	0			
Other alkyl derivatives				
Hydrocarbon				
1-Heptene		0	0	

Table 2 Continued

	Animal			
	M13	F6	F6	F11
Age (day after emergence)	1	0	0	3
Alcohols				
1-Pentanol	0	+	+	+
2,6-Dimethyl-5-hepten-2-ol		++	++	+
(10*E*,12*E*)-14:OH[b]	+			
(10*E*,12*Z*)-14:OH[b]	+			
(10*Z*,12*Z*)-14:OH[b]	+			
(*E*)-12–14:OH[b]	0			
(*E*)-3-Hexenol	+	++	+	
(*Z*)-3-Hexenol			0	
Aldehydes				
1-Heptanal	+	0	+	
1-Nonanal[a]	+	+	+	++
1-Octanal	+	+	+	
(9*E*,11*E*)-14:Al	+			
(E)-2-Hexenal		+	0	
Carboxylic acids				
3-Methyl-butyric acid		0	0	
4-Methyl-pentanoic acid		0	0	
Butyric acid	0	+	0	
Hexanoic acid		0	+	
Nonanoic acid		0	0	
Pentanoic acid		0	0	
Oil extracts				
Jasmine extract		++	++	+
Jasmine oil		+++	++	+
Orange oil (40% germacrene D)[a]				+

In one female (F6) both antennae were used. M = male, F = female (animals of both sexes were numbered consecutively after emegence), 0 = no response, + = weak response (amplitude 0.5—1 mV), ++ = intermediate response (amplitude 1—2 mV), +++ = strong response (amplitude > 2 mV), empty boxes = not tested. Stimulus load was 1 µl (in 9 µl paraffin oil on filter paper) for VOCs and 10 µg (in 10 µl *n*-hexane per filter paper) for putative pheromone components.
[a]VOCs present in *O. stricta.*
[b]Unknown dose of pheromone (see Materials and methods).

responses (Table 2). In the female antenna, many of the VOCs elicited responses (Table 2). Responses with the largest amplitude were elicited by benzyl acetate (2.8 mV) and synthetic jasmine oil (2.6 mV). A weak response to (9*Z*,12*E*)-14:Ac was also observed.

Table 3 Activation of receptor cells from male sensilla trichodea (t) by VOCs (1 μl) and putative pheromone components

	Animal											
	M1	M5	M5	M5	M6	M7	M7	M8	M8	M8	M9	M9
Sensillum	t1	t4	t5	t6	t1	t1	t2	t1	t2	t3	t1	t2
Age (day after emergence)	4	1	1	1	1	1	1	3	3	3	6	6
Terpenoids												
Monoterpenoids												
Hydrocarbon												
Limonene		0		0		0						
Esters												
Linalyl acetate							0					
Terpineol acetate						0	0					
Ketones												
Geranyl acetone		++	++	++	++	+	0					
Neryl acetone						0	+					
Alcohols												
Geraniol		+	++	++	+	++	+					
Linalool		0	++	++		+	+					
Nerol							+					
Terpin-4-ol						0						
Terpineol						0	0					
Aldehyde												
Citral							+					
Sesquiterpenoids												
Hydrocarbons												
β-Caryophyllene[a]		0		0								
Bulnesene							0					
Murolene							0					
Alcohol												
Nerolidol				++								
Aromatics												
Ester												
Benzylacetate						0	0					
Alcohols												
Creosol		+	++	+								
Eugenol							0					
Methyl-eugenol		0										
Aldehyde												
Benzaldehyde							+					
Carboxylic acid												
Benzoic acid		0										

Table 3 Continued

	Animal											
	M1	M5	M5	M5	M6	M7	M7	M8	M8	M8	M9	M9
Sensillum	t1	t4	t5	t6	t1	t1	t2	t1	t2	t3	t1	t2
Age (day after emergence)	4	1	1	1	1	1	1	3	3	3	6	6
Fatty acid derivatives												
(9*E*,11*E*)-14:Ac								+	+	+		0
(9*E*,11*Z*)-14:Ac								++	+	+		+S
(9*Z*,11*E*)-14:Ac								+	+	+		+S
(9*Z*,12*E*)-14:Ac[b]								+++	+++	+++	+++	+++S
(10*E*,12*Z*)-14:Ac								+	+	+		
(10*Z*,12*Z*)-14:Ac								0	0	0		
(*E*)-12–14:Ac								++	++	++		++S
(*Z*)-11-Tetradecen-9-yn:Ac								++	+	+		
(*Z*)-9–14:Ac								0	+	+		+S
Other alkyl derivatives												
Hydrocarbon												
Cyclohexanone	–	+	+									
Alcohols												
2,6-Dimethyl-5-hepten-2-ol							+					
(10*E*,12*E*)-14:OH[b]											++	++L
(10*E*,12*Z*)-14:OH[b]											++	++L
(10*Z*,12*Z*)-14:OH[b]											++	++L
(*E*)-12–14:OH[b]											+	+L
(*Z*)-3-Hexenol		0										
Aldehydes												
(9*E*,11*E*)-14:Al											++	++S
(E)-2-Hexenal		0										
Carboxylic acid												
Pentanoic acid		+										

Several VOCs elicited excitatory responses of intermediate intensity. Solely (9*Z*,12*E*)-14:Ac elicited strong responses of the cell with smaller spike amplitude (S). The cell with the larger spike amplitude (L) was activated by several 14-carbon alcohols. The males (M1-M9) were numbered consecutively after emergence, the sensilla were numbered consecutively within each animal in the order as contacted (e.g. M9 t2 = 2nd sensillum trichodeum of male 9). 0 = no response; + = weak response (slightly increased spike frequency, no receptor potential); ++ = intermediate response (receptor potential 1–10 mV, phasic–tonic nerve impulse response with peak spike frequency 100–200 imp/s); +++ = strong response (receptor potential > 10 mV and/or peak spike frequency > 200 imp/s); – = inhibition of nerve impulses during stimulus application; – – = prolonged inhibition of nerve impulses accompanied by hyperpolarization; ± = combined excitatory and inhibitory response; empty boxes = not tested. For stimulus loads see Table 2.

[a]VOCs present in *O. stricta*.

[b]Unknown dose of pheromone (see Materials and methods).

Physiology: single sensillum recordings

Sensilla trichodea—males

In total, 28 male sensilla trichodea were used for recordings. According to the spike amplitude, usually two (in a few cases three) receptor cells could be distinguished. Seven sensilla were tested with VOCs: some terpenoid and cyclic compounds elicited weak or intermediate, predominantly excitatory responses (Table 3). Five sensilla were tested with a range of pheromone candidates. In all cases, the cell with the smaller

spike amplitude responded to several 14-carbon acetates and to (9*Z*,12*E*)-tetradecadienylaldehyde, with the strongest responses being elicited by (9*E*,11*E*)-14:Ac. The cell with the larger spike amplitude gave equal responses of intermediate intensity to the three geometrical isomers of 10,12-tetradecadienol: *Z,Z-, E,E-* and *E,Z-* (Table 3).

A further 16 male s. trichodea, each containing a cell strongly and selectively responding to (9*Z*,12*E*)-14:Ac, were used to measure the dose-response dependence of the maximum receptor potential amplitude and the peak spike frequency (Figure 3A). Responses significantly higher than control (P = 0.0111 for receptor potential amplitude, $P < 0.0001$ for nerve impulse frequency, paired t-test) were elicited already by a 10^3-fold dilution of the original (9*Z*,12*E*)-14:Ac stock solution, the threshold being apparently close to a 10^4-fold dilution. The relatively high nerve-impulse responses to control stimuli (Figure 3A, lower graph) were probably due to contamination by pheromone from females which were used for experiments within the same setup.

Sensilla trichodea—females

Recordings were performed on 36 female s. trichodea (Table 4); these were about half as long as the corresponding male sensilla. Usually two receptor cells were present in each sensillum according to their different spike amplitudes.

Some of the tested terpenoid and aromatic compounds elicited responses in those cells. In three sensilla, quite unspecific inhibitory responses were observed. Excitatory responses were repeatedly elicited by nerolidol, geraniol, neryl acetate, synthetic jasmine oil, jasmine extract and orange oil. Most of the receptor cells were quite specific and responded to only one or few tested compounds. Most abundant was a cell (n = 6) present in 15% of the female s. trichodea and responding selectively and specifically to nerolidol, with a threshold stimulus dose at $\sim 10^{-3}$ µl (= ca. 1 µg) per filter paper (Figure 3B). In one case (Table 4, F4/t7), a nerolidol-sensitive cell responded with a similar intensity and even lower threshold to geraniol. The other five nerolidol-sensitive cells responded to geraniol only weakly or not at all (Table 4). The dose–response dependence of the maximum receptor potential amplitude and the peak spike frequency of the six nerolidol-sensitive cells is shown in Figure 3B. The relatively high nerve-impulse responses to control stimuli (Figure 3B, lower graph) result from previous activation of the cells with high doses of several of the tested compounds before the dose–response dependencies were measured.

The receptor cells of the female s. trichodea did not respond to saturated and unsaturated aliphatic alcohols, aldehydes and acids tested. A single weak response to the

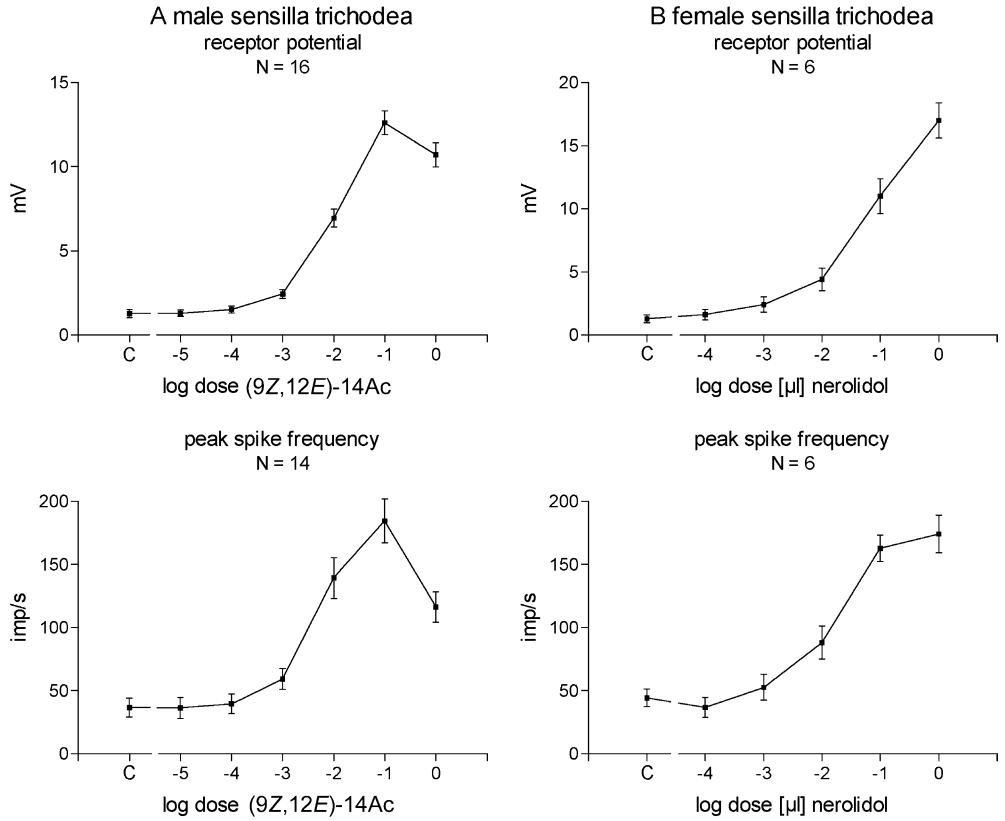

Figure 3 Dose–response dependencies of the receptor potential amplitude (above) and peak spike frequency (below) registered in the male pheromone sensitive cell **(A)** and the female nerolidol-sensitive cell **(B)**. Concentration of the (9*Z*,12*E*)-14:Ac pheromone stock solution was not known, the dilution series was by factor 10. C, control (clean filter paper). Means ± SE are shown.

Table 4 Activation of receptor cells from female sensilla trichodea (t) by VOCs (1 μl)

Animal	F1	F2	F2	F2	F2	F3	F3	F3	F4	F4	F4	F4	F4	F4	F4	F5	F5	F7	F7	F7	F8	F8	F9	F9	F9	F9	F10	F11	F11	F11	F12	F12	F12	F13	F13	F13
Sensillum	t1	t1	t2	t3	t4	t1	t2	t3	t1	t2	t3	t4	t5	t6	t7	t1	t2	t1	t2	t3	t1	t2	t1	t2	t3	t4	t1	t1	t2	t3	t1	t2	t3	t1	t2	t3
Age (day after emergence)	1	2	2	2	2	1	1	1	2	2	2	2	2	2	2	3	3	5	5	5	2	2	3	3	3	3	3	3	3	3	2	2	2	3	3	3
Terpenoids																																				
Monoterpenoids																																				
Hydrocarbons																																				
α-Pinene[a]						0	0	0	0	0	0	0			0			0	0	+S	0		0	0			0	0	0				0	0	0	
α-Terpinene[a]						0	0		0	0		0																								
β-Pinene						+	0	0	0	0	0	0		0	0			0	0	0	0		0				0	0	0				0	0	0	
Camphene						0	0		0	0		0																								
δ-3-Carene						0	0		+L	0		0																								
γ-Terpinene						0	0		0	0		0																								
Limonene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	+S	0	++S	0	0		0	0	0	0	0			0	0	0	0
Myrcene						0	0		0	0		0						0	0	0	0						0	0	0				0	0	0	
Esters																																				
Citronellyl acetate						0	0		0	0	0	0	0	0	+		0	0	0	0	0						0	0	0				0	0	0	
Linalyl acetate						0	0	0	0	0	0	0	0	0	0		0	0	0	0	0						0	0	0				0	0	0	
Neryl acetate						0	+	0	++LS	0	0	++	0	++	0			0	0	0	0						0	+	0				0	0	0	
Terpineol acetate						0	0	–	0	0	0	0					0	0	0	0	0						0	0	0				0	0	0	
Ketones																																				
cis-Verbenone						0	0		0	0		0						0	0	0	0						0	0	0				0	0	0	
Geranyl acetone		0	0	0	0	++	0	–	+L	0	0	0	0	0	++		0	0	0	0	0						0	0	–				0	0	0	
Neryl acetone						0	0	–	0	0	0	0	0	0	+		0	0	0	0	0						0	0	0				0	0	0	
Thujone						0	0			0		0																								
Alcohols																																				
Citronellol						0	0	0	0	0	0	0	0	0	0			0	0	+S	0	++S	0	0		0	0	0	0				0	0	0	
Geraniol		0	0	0	0	++	0	–	0	0	0	0	0	0	+++	+	0	0	0	++S	0	++S	0	0	0	0	0	0	–	0	0	0	+	0	+	0
Linalool		0	0	0	0	0	0	–	0	0	0	0	0	0	0		0	0	0	+S	0	++S	0	0	–	0	0	0	–				0	0	0	0
Nerol						0	0	0	+L	0	0	0	0	0	0			0	0	+S	0						0	0	0				0	+	0	
Terpin-4-ol						0	0		0	0		0						0	0	0	0						0	0	–				0	0	0	
Terpineol						0	0	–	0	0	0	0		0				0	0	+S	0	0					0	0	–				0	0	0	
Thujyl alcohol						0	0		0	0		0																								
Aldehyde																																				
Citral						+	0	–	0	0	0	0	0	0				0	0	++S	0	0	0	0	0	0	0	0	–	+	0	0	0	0	0	0
Sesquiterpenoids																																				
Hydrocarbons																																				
α-Caryophylene[a]						0	0	0	0	0		0						0	0	0	0						0	0	0				0	0	0	
β-Caryophyllene[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0		0	0		0	0	0	0				0	0	0	

Table 4 Continued

	Animal																																			
	F1	F2	F2	F2	F2	F3	F3	F3	F4	F4	F4	F4	F4	F4	F4	F5	F5	F7	F7	F7	F8	F8	F9	F9	F9	F9	F10	F11	F11	F11	F12	F12	F12	F13	F13	F13
Sensillum	t1	t1	t2	t3	t4	t1	t2	t3	t1	t2	t3	t4	t5	t6	t7	t1	t2	t1	t2	t3	t1	t2	t1	t2	t3	t4	t1	t1	t2	t3	t1	t2	t3	t1	t2	t3
Age (day after emergence)	1	2	2	2	2	1	1	1	2	2	2	2	2	2	2	3	3	5	5	5	2	2	3	3	3	3	3	3	3	3	2	2	2	3	3	3
Bulnesene						0	0	0	0	0		0																								
Guajene						0	0		0	0		0																								
Murolene						0	0	0	0	0		0																								
Alcohol																																				
Nerolidol	0	0	0	0	0	+++	0	0	0	0	0	0	0	0	+++	+++	0	0	0	0	0	+++S	0	0	–	0	0	0	0	+++	+++	0	0	0	0	0
Diterpenes																																				
Alcohol																																				
Isophytol						0	0		0	0		0						0	0	0	0						0	0	0				0	0	0	
Aromatics																																				
Hydrocarbon																																				
p-Cymene						0	0		+L	0		0						0	0	0	0		0				0	0	0				0	0	0	
Ester																																				
Benzylacetate						0	+	–	++LS	0	0	0	0	0			0	0	0	0	0		0				0	0	0				0	0	0	
Alcohols																																				
1-Phenylethanol						0	0		0										0									0								
2-Phenylethanol						0	0		0										+									0								
Creosol	0	0	0	0	0	0	0		0			0							0									0								
Eugenol						0	0	–	+S	0	0	0	0	0	0			0	0	0	0	0					0	0	0				0	0	0	
Methyl-eugenol		0	0	0	0	0	0	–	0	0	0	0						0	0	0	0	0					0	0	0				0	0	0	
Aldehydes																																				
Benzaldehyde						0	0	–	0	0		0	0	0	0			0	0	0	0						0	0	0				0	0	0	
Phenyl-acetaldehyde						0	0		0										0									0								
Carboxylic acid																																				
Benzoic acid									0	0		0	0	0																						
Fatty acid derivatives																																				
Ester																																				
(9*Z*,12*E*)-14:Ac[b]																0	0		0	0	0	0	0	0	0					0	0	+	0	0	0	
Other alkyl derivatives																																				
Hydrocarbons																																				
Cyclohexanone	0	0	0	0	0																															
Cyclopropanone	0																																			
Alcohols																																				
1-Heptenol						0			0	0		0								0	0				0		0	0	0				0	0	0	0
1-Pentanol						0	0		0	0		0						0	0		0	0		0	0		0	0	0			0	0	0	0	0
2,6-Dimethyl-5-hepten-2-ol						0	0	–	0	0	0	0	0	0	0			0	0	++S	0	0			–	0	0	0	–	0	0	0	0	0	0	0

Table 4 Continued

	Animal																																		
	F1	F2	F2	F2	F2	F3	F3	F3	F4	F4	F4	F4	F4	F4	F4	F5	F5	F7	F7	F7	F8	F8	F9	F9	F9	F9	F10	F11	F11	F11	F12	F12	F12	F13	F13	F13
Sensillum	t1	t1	t2	t3	t4	t1	t2	t3	t1	t2	t3	t4	t5	t6	t7	t1	t2	t1	t2	t3	t1	t2	t1	t2	t3	t4	t1	t1	t2	t3	t1	t2	t3	t1	t2	t3
Age (day after emergence)	1	2	2	2	2	1	1	1	2	2	2	2	2	2	2	3	3	5	5	5	2	2	3	3	3	3	3	3	3	3	2	2	2	3	3	3
(*E*)-3-Hexenol						0			0	0		0								0	0				0		0	0	0			0	0	0	0	0
(*Z*)-3-Hexenol	0	0	0	0	0	0			0	0		0							0	0	0				0		0	0	0				0	0	0	0
Aldehydes																																				
1-Heptanal						0			0	0		0							0	0	0			0	0		0	0	0				0	0	0	0
1-Nonanal[a]																		0	0		0	0			0		0	0	0	0	0		0	0	0	0
1-Octanal																			0	0	0				0		0	0	0	0	0		0	0	0	0
E-2-Hexenal	0	0	0	0	0	0			0	0		0								0	0				0		0	0	0				0	0	0	0
Carboxylic acids																																				
3-Methyl-butyric acid		0	0	0	0	0			0										0									0								0
4-Methyl-pentanoic acid		0	0	0	0	0			0																											0
Butyric acid						0			0																											0
Hexanoic acid		0	0	0	0	0			0																											0
Nonanoic acid						0			0																											0
Pentanoic acid		0	0	0	0	0			0																											0
Oil extracts																																				
Jasmine extract						0			0	0		0						0	0	++L	0	++S	0	0	–	0	0	0	0			0	0	0	0	0
Jasmine oil						0	0		0	0	0	0		0				0	0	+++S	0	++S					0	0	0			0	0	0	0	
Orange oil (40% germacrene D)[a]																							0	0	0	++	0	0	0	0	0	0	+	0	0	++

The single cells were predominantly specialists, responding, if at all, to one or a few compounds. In some sensilla, more than one cell responded to the volatiles, and the cells could be distinguished by spike amplitude: S, smaller spike; L, larger spike. The females (F1–F13) were numbered consecutively after emergence. For further explanations see Table 3.

putative pheromone component (9*Z*,12*E*)-14:Ac was observed in a female s. trichodeum (Table 4, F12/t2).

Sensilla auricillica and basiconica

In total, 14 male and 8 female s. auricillica or s. basiconica were tested with a range of VOCs. No physiological or morphological differences between the sexes were observed. The sensilla contacted by the electrode were predominantly ear-shaped (s. auricillica) and were located at the border to the part of the antenna covered by scales, or even below the scales. A few very short hairs which seemed not to be flattened (s. basiconica) were also contacted, but the two morphological types were difficult to distinguish under the stereomicroscope. As physiological differences were not apparent either, they were all evaluated within one group (Table 5).

Usually up to three receptor cells were localized in these sensilla according to different spike amplitudes. They responded regularly with excitation or inhibition (Figure 4) to several terpenoid compounds (e.g. linalool, geraniol, limonene, citral, dimethyl-heptenol, jasmine extract and orange oil) as well as to aliphatic alcohols, aldehydes and acids (Table 5). Many of the cells were apparently generalists responding strongly to several related compounds. Strong responses to nonanal, octanal and heptanal (Figure 5) were recorded from ~ 50% of sensilla tested with these compounds.

Sensilla coeloconica

Five s. coeloconica from two male moths could be contacted and were tested with several saturated and unsaturated aliphatic alcohols, aldehydes and acids. Usually at least three receptor cells were present according to the spike amplitudes. The receptor cells of the coeloconic sensilla did not respond to alcohols, while responses to aldehydes and acids were regularly obtained (Table 6 and Figure 6). Each cell responded

Table 5 Activation of receptor cells from male and female sensilla auricillica/basiconica (ab) to VOCs (1 µl)

	Animal																						
	M2	M3	M3	M10	M11	M12	M14	M18	M19	M21	M21	M22	M22	M23	F2	F2	F4	F4	F5	F8	F12	F12
Sensillum	ab1	ab1	ab2	ab1	ab1	ab1	ab1	ab1	ab1	ab1	ab2	ab1	ab2	ab1	ab1	ab2	ab1	ab2	ab1	ab1	ab1	ab2
Age (day after emergence)	1	2	1	9	11	11	1	3	4	3	3	4	4	3	2	2	2	2	3	2	2	2
Terpenoids																						
Monoterpenoids																						
Hydrocarbons																						
α-Pinene[a]						0	0	+									0		0			
β-Pinene[a]						0	0										0		0			
γ-Terpinene				0																		
Limonene	++	++	++	0		0	0	++	0	0	0		0	+	++	+	0	0	0			0
Ester																						
Terpineol acetate				0																		
Ketones																						
Geranyl acetone			±												0	0						
Thujone																			0			
Alcohols																						
Citronellol							0	+		0			0				–					
Geraniol		++	++	0		0	0	++	0	0	0		0	0	+++	+++	–					0
Linalool		++	++	0		0	0	+		0	++		0		++	+	0					0
Nerol																	0					
Terpineol				0		0											0	0				
Aldehyde																						
Citral						+++	0	++	0	+	+++		0	++			–		0			0
Sesquiterpenoids																						
Hydrocarbons																						
α-Caryophyllene[a]				0		0																
β-Caryophyllene[a]	0	+	+	0		0	0	+		0					0		0		0			
Alcohol																						
Nerolidol		0	–	0		0	0	++	0	0	0		0		0	0	–					0
Aromatics																						
Hydrocarbon																						
p-Cymen						0	0												0			
Alcohols																						
1-Phenylethanol																	0					
Creosol			–												0							
Eugenol				0		0											–	++	0			
Methyl-eugenol				++		0																
Aldehydes																						
Benzaldehyde																	0					

Table 5 Continued

	Animal																					
	M2	M3	M3	M10	M11	M12	M14	M18	M19	M21	M21	M22	M22	M23	F2	F2	F4	F4	F5	F8	F12	F12
Sensillum	ab1	ab1	ab2	ab1	ab1	ab1	ab1	ab1	ab1	ab1	ab2	ab1	ab2	ab1	ab1	ab2	ab1	ab2	ab1	ab1	ab1	ab2
Age (day after emergence)	1	2	1	9	11	11	1	3	4	3	3	4	4	3	2	2	2	2	3	2	2	2
Alkyl derivatives																						
Hydrocarbons																						
1-Heptene				0	0	0	0		0			0		++						0		
Cyclohexanone			–												0							
Alcohols																						
1-Pentanol				++	++	0	0		0			++	++	++						0	0	0
2,6-Dimethyl-5-hepten-2-ol								++	0	0	++		0									
(*E*)-3-Hexenol				+	0	0	0		0	0		++		++						+SL		
(*Z*)-3-Hexenol	+	0	0	+	0	++	0		0			++		++	0	0	0		0	0		
Aldehydes																						
1-Heptanal				+		+++	0		0		+++	+++		++						++S	++	++
1-Nonanal[a]				0		+++	0		0		+++	+		++					0	+++S	+++	+
1-Octanal				0		+++	0		0		+++	+++		++						++S	+++	++
(*E*)-2-Hexenal	0	0	0	+	0	++	0		0		++	0		++	0	+	0		0	0	0	0
Carboxylic acids																						
3-Methyl-butyric acid							+							++								
4-Methyl-pentanoic acid							+							++								
Butyric acid							+						0	++								
Hexanoic acid						++	+							++								
Nonanoic acid						++	0				++			++							++	
Pentanoic acid							+							++								
Oil extracts																						
Jasmine extract											+++		0	++								
Jasmine oil													++						0			
Orange oil (40% germacrene D)[a]								++	0		0		0	+++								++

The receptor cells were predominantly generalists, responding to several terpenoids and green leave volatiles. For further explanations see Table 3.

best to a single compound, and much less or not at all to related compounds. In some cells, aldehydes elicited stronger responses than acids of the same chain length (Figure 6).

Discussion

Putative pheromone components

In Phycitinae species closely related to *C. cactorum* the pheromone components known so far are predominantly 14-carbon acetates, alcohols and aldehydes with two double bonds at positions 9 and 12, with (9*Z*,12*E*)-14:Ac being one of the most common components within this group of moths (Arn *et al.*, 1992; Witzgall *et al.*, 2004). According to the responses obtained from receptor cells of male s. trichodea of *C. cactorum*, (9*Z*,12*E*)-14:Ac seems to be one of the pheromone components of *C. cactorum*, specifically exciting the pheromone receptor cell with a smaller spike amplitude. Although the absolute concentration of this compound was not known, the fact that this compound was effective even if diluted by a factor of 10^4 supports the idea that (9*Z*,12*E*)-14:Ac is indeed a pheromone component of *C. cactorum*.

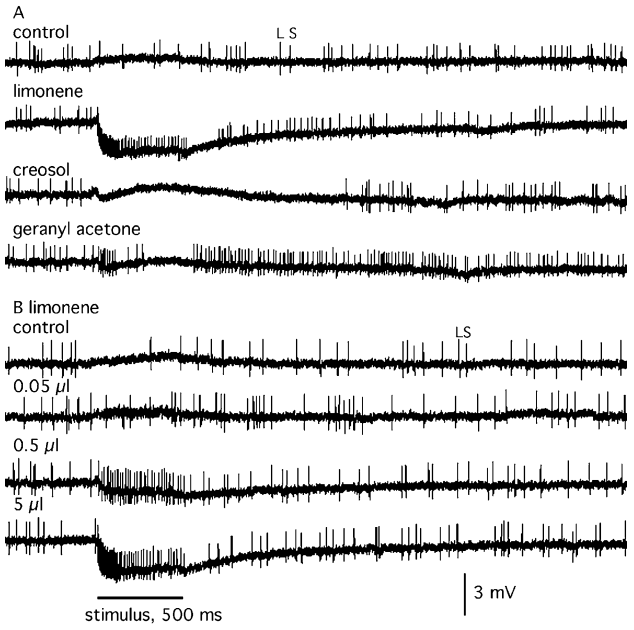

Figure 4 Responses recorded from a singe male sensillum basiconicum (Table 5, M3/ab2). **(A)** The receptor cell with the smaller spike amplitude (S) was excited by limonene (5 µl) and inhibited by creosol (1 µl). Geranyl acetate (1 µl) elicited a mixed excitatory/inhibitory response. **(B)** Responses of the same cell to increasing dose of limonene. L, spike with large amplitude.

The cell with the larger spike amplitude responded weakly to (10*Z*,12*Z*)-tetradecadienol, (9*E*,12*E*)-tetradecadienol and (10*E*,12*Z*)-tetradecadienol. (9*Z*,12*E*)-Tetradecanol was not available for testing, but it may be another candidate pheromone component. Further quantitative studies are necessary.

The male pheromone-sensitive cells were excited additionally by several terpenoid compounds. This might be a general, receptor-independent effect similar to the inhibition of pheromone-sensitive cells of *Antheraea pernyi* by geraniol (Schneider *et al.,* 1964) or the inhibition of pheromone sensitive cells of *Bombyx mori* by linalool (Kaissling *et al*., 1989; Pophof and van der Goes van Naters, 2002). A synergism between pheromones and plant odorants at the receptor level, as observed in *Helicoverpa zea* (Ochieng *et al*., 2002), was not studied in this paper, but it could occur as a consequence of consecutive stimulation with pheromones and other odorants.

The putative pheromone component (9*Z*,12*E*)-14:Ac elicited EAG responses also in female antennae (Table 2). These responses were weak, but comparable to EAGs elicited by nerolidol, which excited a considerable subset of receptor cells localized in female trichoid sensilla. Therefore, it seems probable that pheromone sensitive receptor cells occur on female antennae. Pheromone autodetection was observed in females of several moth species (Ljungberg *et al*., 1993; Todd and Baker, 1993; Schneider *et al*., 1998). For this reason, (9*Z*,12*E*)-14:Ac was tested also on female s. trichodea, but not in the other sensillum types, as in most moth species pheromone sensitive cells are localized exclusively in s. trichodea. Only one weak single-cell response to (9*Z*,12*E*)-14:Ac was observed in a single s. trichodeum (Table 4).

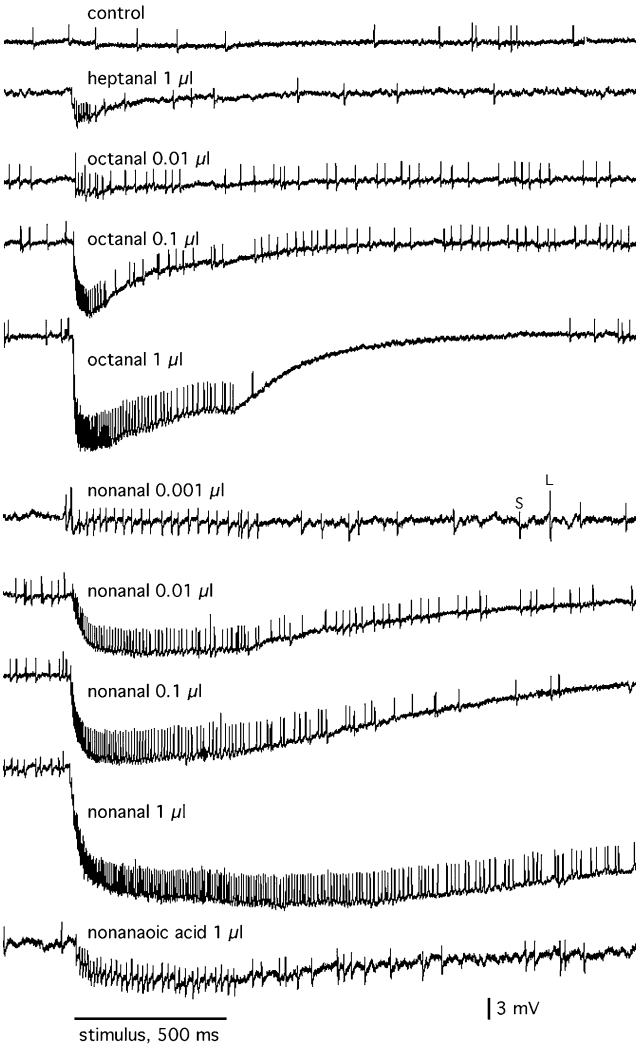

Figure 5 Responses of cells from a sensillum auricillicum of a female (Table 5, F12/ab1) to aliphatic aldehydes and acids. The cell with the smaller spike amplitude (S) responded weakly to heptanal and nonanoic acid and strongly to octanal and nonanal, the latter being the best stimulus. L, spike with large amplitude.

VOCs

Eight VOCs were identified in the headspace of the prickly pear *O. stricta*, a major host plant of *C. cactorum*, using GC-MS (Table 1). The present data do not show striking differences between VOCs of *O. stricta* and plants with C_3 or C_4 metabolism. The origin of plant constituents from CAM metabolism is usually proven by isotope ratio mass spectrometry (Preston *et al*., 2003). Several C_6 and C_9 aroma compounds were characterized by Weckerle *et al.* (2001) from extracts of *Opuntia ficus indica* cactus pear fruits using isotope ratio mass spectrometry and they showed that especially 1-hexanol, (*E*)-2-hexenol, (*E*)-2-nonenol and

Table 6 Activation of receptor cells from male sensilla coeloconica (c) by aliphatic acids and aldehydes

	Animal				
	M7	M7	M7	M20	M20
Sensillum	c1	c2	c3	c1	c2
Age (day after emergence)	1	1	1	3	3
Alkyl derivatives					
Hydrocarbon					
1-Heptene	0			0	
Alcohols					
1-Pentanol				0	
(*E*)-3-Hexenol	0			0	
(*Z*)-3-Hexenol	0			0	
Aldehydes					
1-Heptanal	0			+++	+
1-Nonanal[a]				0	++
1-Octanal				++	+
(*E*)-2-Hexenal	0			+	+
Carboxylic acids					
3-Methyl-butyric acid			+++	+	0
4-Methyl-pentanoic acid		0	0	0	0
Butyric acid			0	0	+
Hexanoic acid		+++	0	++	+
Nonanoic acid		0	0	0	+
Pentanoic acid			0	++	+

For explanations see Table 3.

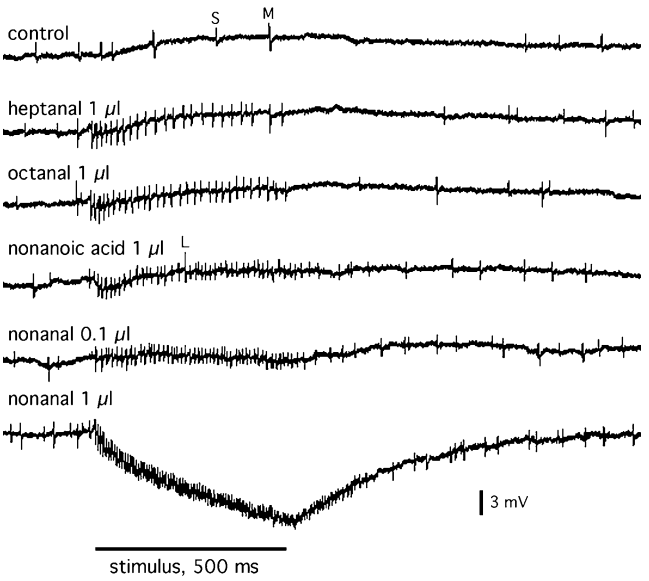

Figure 6 Responses recorded from a single male s. coeloconicum (Table 6, M20/c2) containing three receptor cells. The cell with the intermediate spike amplitude (M) responded best to nonanal; nonanoic acid, octanal and heptanal activated this cell only weakly. L, spike with large amplitude; S, spike with small amplitude.

(2*E*,6*Z*)-nonandienol originate from the CAM metabolism. The only compound which has been found both in the headspace of *O. stricta*, and in extracts from fruits of the cactus pear, was nonanal. Nonanal has also been reported from non-platyopuntia cacti species in moderately low concentrations (Kaiser and Nussbaumer, 1990; Kaiser and Tollsten, 1995).

Six of the VOCs identified from *O. stricta* were tested electrophysiologically on the antennae and sensilla of both sexes of *C. cactorum*: the terpenoids α-pinene, β-pinene, α-caryophyllene, β-caryophyllene and germacrene D from orange oil elicited rather low responses in a few cells of female s. trichodea (Table 4) and male *s.* auricillica/basiconica (Table 5), as well as in female EAGs (Table 2). Nonanal seems to be a strong activator of a large proportion of receptor cells localized in male and female s. auricillica/basiconica and s. coeloconica (Tables 5 and 6). Therefore, nonanal, and possibly other related C_9 compounds, might represent candidate attractants of *C. cactorum* and should be tested behaviorally.

Schneider *et al.* (1964) divided olfactory receptor cells into two groups: specialists, responding with high sensitivity to a narrow spectrum of compounds, which is identical for all cells of a certain cell type, and generalists, responding with lower sensitivity to different, but overlapping spectra of compounds. According to this classification, the s. trichodea of *C. cactorum* females house predominantly specialists (Table 4), as do trichoid sensilla of several other moth species, e.g. *Trichoplusia ni* (Todd and Baker, 1993) or *Spodoptera littoralis* (Anderson *et al.*, 1995). The most abundant and best characterized receptor cell of *C. cactorum* specialized to a floral odor was the nerolidol-sensitive cell occurring in ~15% of the female trichoid sensilla. Terpenes and terpene-alcohols, including nerolidol, are common odorants of moth pollinated flowers, including several cactus species (Kaiser and Tollsten, 1995). Accordingly, receptor cells specialized to such odorants occur on the antennae of several moth species. In three heliothine species, the most abundant specialist receptor responding to VOCs of plant origin was a cell specialized to germacrene D present in ~50% of the female sensilla (Stranden *et al.*, 2003). In *Bombyx mori* females, all trichoid sensilla are identical and contain two specialist receptor cells that respond best to 2,6-dimethyl-5-hepten-2-ol and benzoic acid (Priesner, 1979; Heinbockel and Kaissling, 1996).

Receptor cells of s. auricillica or s. basiconica contain specialists and generalists, responding to terpenes as well as to green leaf volatiles (Table 5). This is in accordance with recordings from the basiconic sensilla of *Spodoptera littoralis*

(Noctuidae), which contain cell types specialized to certain terpenes (e.g. geraniol, α-humulene and β-caryophyllene) besides generalists responding to a broad range of green leaf volatiles (Anderson *et al*., 1995) and recordings from s. auricillica of *Scoliopteryx libatrix* (Noctuidae) which revealed receptor cells responding to δ-3-carene, (+/−)-linalool, α-pinene and green leaf volatiles (Anderson *et al.,* 2000).

Similarly to s. coeloconica of *Bombyx m*ori (Pophof, 1997), the receptor cells of coeloconic sensilla of *C. cactorum* responded to aliphatic acids and aldehydes (Table 6). In contrast to *B. mori*, the cells of *C. cactorum* could distinguish between acids and aldehydes of the same chain length and responded stronger to aldehydes.

There was a certain overlap in the specificity of receptor cells housed in morphologically distinct sensillum types. Cells specialized to certain terpenoid compounds occurred in s. trichodea as well as in s. auricillica/basiconica, cells responding strongly to aldehydes occurred in s. auricillica/basiconica and s. coeloconica. However, no overlap between s. trichodea and s. coeloconica was found; cells from female s. trichodea never responded to aliphatic compounds tested in this study.

In *C. cactorum*, cells of many female sensilla did not respond to any of the tested compounds (Tables 3 and 4). Therefore, the odorants activating these cells remain undiscovered. Furthermore, only a small proportion of sensilla was contacted, and due to missing morphological data the total number of contacted neurons remains unknown. Weak EAG responses to compounds activating strongly specialized receptor cells (e.g. nerolidol, nonanal, Table 2) indicate that strong activation of a certain small subset of sensilla is not reflected by a large EAG- amplitude (see also Wibe, 2004). Therefore, in *C. cactorum* EAG seems not to be a suitable method to search for volatiles perceived by this moth species. On the other hand, strong activators of certain cell types might be overlooked by GC-MS analysis due to their low concentrations. Therefore, a search for behaviorally active VOCs could be continued more productively by GC linked to single sensillum recordings (GC-SCR), as used recently in several other moth species (e.g. Røstelien *et al.,* 2000a,b; Stranden *et al*., 2002, 2003). Also, compounds present in trace amounts, which may be biologically active, may be pursued in future work using more sensitive mass spectrometric detection.

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