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Functional Characteristics of a Tiny but Specialized Olfactory System: Olfactory Receptor Neurons of Carrot Psyllids (Homoptera: Triozidae)

Lina Kristoffersen[1], Mattias C. Larsson[2] and Olle Anderbrant[1]

[1]Department of Ecology, Chemical Ecology and Ecotoxicology, Lund University, Sölvegatan 37, SE-223 62 Lund, Sweden and [2]Department of Crop Protection Biology, Chemical Ecology, Swedish University of Agricultural Sciences, SE-230 53 Alnarp, Sweden

Correspondence to be sent to: Mattias C. Larsson, Department of Crop Protection Biology, Chemical Ecology, Swedish University of Agricultural Sciences, Box 102, SE-230 53 Alnarp, Sweden. e-mail: mattias.larsson@ltj.slu.se

Abstract

With only ~50 olfactory receptor neurons (ORNs), the carrot psyllid *Trioza apicalis* (Homoptera: Psylloidea) may have the smallest olfactory system described in adult Neopteran insects. Using single sensillum recordings (SSR) and gas chromatograph–linked SSR, we characterized 4 olfactory sensilla forming a distinct morphological type, which together house ~25% of all ORNs. We recorded responses to extracts and single constituents from *Daucus carota* ssp. *sativus*, from the conifers *Picea abies*, *Pinus sylvestris*, and *Juniperus communis*, as well as from male and female *T. apicalis*. Receptor neurons were highly selective; only 9 compounds in total elicited repeatable responses, and each neuron responded to at most 3 individual compounds. Chemical profiles of carrot and conifers showed significant overlap, with 4 out of 9 electrophysiologically active compounds occurring in more than one type of extract, but a carrot-specific compound elicited the most repeated responses. We identified 4 tentative neuron classes and found a rather high degree of neuronal redundancy, with 1 neuron class present in 3 and another present in all 4 of the sensilla, respectively.

Key words: GC-SSR, host plant, olfaction, sensillum, single sensillum recording, *Trioza apicalis*

Introduction

Most insects rely on their sense of smell for host and mate location (Schoonhoven et al. 2005). Molecular and neuroanatomical studies have shown that adult insects typically perceive their olfactory environment by means of rather few (ca., 40–160) functional types of olfactory receptor neurons (ORNs), each defined by the expression of a single unique olfactory receptor protein (Rospars 1988; Vosshall et al. 2000). In vivo, electrophysiological recordings from single ORNs in insects suggest that many or most of these have high affinity for a narrow range of specific compounds that guide olfactory orientation. This feature is most apparent in the case of ORNs tuned to single sex pheromone components in moths and many other insect groups, but examples of selectively tuned ORNs for host and nonhost volatiles are also abundant (Anderson et al. 1995; Hansson et al. 1999; Larsson et al. 2001; for a general discussion, see Bruce et al. 2005; Larsson and Svensson 2005). The high selectivity displayed by insect olfactory systems has been heavily exploited to screen biological extracts for behaviorally relevant compounds based on combined gas chromatography and electrophysiological techniques such as electroantennography or single sensillum recordings (SSR) (Stensmyr et al. 2001; Nojima et al. 2003; Wibe 2004; Röstelien et al. 2005; Ulland et al. 2008).

In insects, the total number of ORNs usually exceeds the number of functional types by several orders of magnitude. Fruit flies have approximately 1200 ORNs per antenna (Stocker 1994), whereas in male moths this number can be as high as tens or hundreds of thousands, most of which are tuned to the female sex pheromone (Rospars 1988). Some insects, on the other hand, appear completely anosmic or have greatly reduced olfactory systems, for example, the explicitly acoustic cicadas or visually oriented dragonflies (Strausfeld et al. 1998). Most larvae of holometabolous insects, presumably facing less demanding tasks, also have reduced olfactory systems compared with conspecific adults. This has been most thoroughly characterized in the *Drosophila* larva, which has 21 functionally unique neurons in its bilaterally symmetrical olfactory organ (Kreher et al. 2005). However, some insects have comparatively simple olfactory systems even though they apparently depend on long-range olfactory orientation. Among these are the Homoptera, including aphids and psyllids, whose life cycles may be

complex and often involve host alternation, which requires the capacity to locate and recognize different host plants. Even for Homoptera, the olfactory systems of the Psylloidea seem particularly minute (Chapman 1982; Kristoffersen et al. 2006). Here, we present a study of the plant alternating carrot psyllid *Trioza apicalis* Förster (Homoptera: Psylloidea) with the 2-fold aim of studying the capacity of its small olfactory system to cope with complex life history traits and to find attractants for monitoring of this economically important pest species. *Trioza apicalis* is a pest insect in carrot fields of northern and central Europe. Its life cycle is successfully completed only on carrot, *Daucus carota* ssp. *sativus*, but it may temporarily be sustained on other Apiaceae (Rygg 1977; Valterová et al. 1997). The new adult generation migrates in autumn to conifers, Norway spruce *Picea abies*, Scots pine *Pinus sylvestris*, and juniper *Juniperus communis*, where they pass the winter (Láska 1974; Rygg 1977; Kristoffersen and Anderbrant 2007).

The *T. apicalis* antenna is sparsely equipped with sensilla, containing less than 50 ORNs in total (Kristoffersen et al. 2006). Five different types of putative olfactory sensilla have been described for *T. apicalis*, 2 of which were novel findings (Kristoffersen et al. 2006). There are 4 cuticular cavities positioned laterally on the antenna, and inside each cavity 2 sensilla are located, one on top of the other, neither visible from the outside. The upper and larger one of these sensilla is a mushroom-shaped, thin-walled sensillum with an extensively porous surface, innervated by 3 ORNs, which branch within the sensillum (Kristoffersen et al. 2006). Here, we have investigated the function of this particular sensillum type.

A primary goal of this study was to confirm an olfactory function of the large cavity sensilla, as suggested by Kristoffersen et al. (2006), and to determine if these sensilla have a specific olfactory task, such as discrimination between different plant odor bouquets or intraspecific communication. In this context, we wanted to determine whether active odor components were the same in carrot and conifers. There is only limited data suggesting possible pheromonal activity in psylloids (Soroker et al. 2004), but no candidate compounds have been suggested. Secondly, as these 4 sensilla make up a clearly defined morphological type that comprises ~25% of the ORNs, we wanted to investigate the degree of redundancy and specificity in this olfactory system. In other words, what combinations of functional types of neurons do these sensilla contain, and what is the degree of specialization on the single neuron level?

Material and methods

Insects

Insects were reared continuously on carrot plants (*D. carota*, cultivar Nestor) in a climate chamber under a 20:4 h light: dark cycle, 20:15 °C temperature regime, and 40% relative humidity. The culture originates from *T. apicalis* collected in the field in Finland and southern Sweden.

Stimuli

Hexane extracts of carrot, *D. carota*, and the 3 main winter shelter plants, *P. abies*, *P. sylvestris*, and *J. communis*, were produced by extracting 1 g of leaf material or needles in 1 ml redistilled hexane for 3 h at room temperature. The plant material was then discarded and the solvent retained. Extracts were concentrated by evaporation to approximately half their volume. We used carrot leaves from the same cultivar of plants used in our psyllid culture. Conifer branches were cut fresh and brought to the laboratory immediately before extraction. Insect extracts were made by the same procedure, using whole-body extracts of 250 live *T. apicalis* to 0.5 ml redistilled hexane. A mixed extract made from both sexes was used initially, later supplanted by separate extracts from males and females.

Stimulus cartridges were prepared by applying 10 µl of the stimuli, whether extracts or dilutions of single compounds, on filter paper strips inserted into glass Pasteur pipettes. Hexane- or paraffin-loaded pipettes were used as blank stimuli. Single compounds identified from responses in gas chromatograph–coupled SSR (GC-SSR) and subsequent gas chromatograph–coupled mass spectrometry (GC-MS) were used for dose–response experiments. Four doses of nonanal (Acros, Morris Plains, NJ; 98%), terpinene-4-ol (W. Francke, University of Hamburg), terpinolene (W. Francke, University of Hamburg) and 5 doses of (Z)-3-hexenal (50% in Triacetin; Sigma-Aldrich, St. Louis, MO) from 10 ng to 10 or 100 µg were tested. For (Z)-3-hexenal, paraffin oil (Merck, Whitehouse Station, NJ) was used as solvent for dilutions; for the other compounds, we used redistilled hexane. A stimulus pipette containing a fresh 0.5-g carrot leaf, cut the same morning, was used as a positive control of the preparations before recordings were initiated, as we soon discovered that all tested sensilla responded to carrot.

Electrophysiological techniques

Live *T. apicalis* were inserted into 100 µl disposable plastic micropipette tips, with the head protruding at the tip. The pipette tip was fixed in dental wax on a glass slide with the insect's ventral side facing up and the antennae positioned on an elevated glass cover slip covered with double-sided sticky tape. These preparations could remain useful for several hours. Electrophysiological recordings were performed by means of tungsten microelectrodes, electrolytically sharpened in a saturated KNO_2 solution, which were inserted into single sensilla. The recording electrode was connected to a DC-3K micromanipulator with a Piezo translator (PM 10) (Märzhäuser, Wetzlar-Steindorf, Deutschland). For data acquisition, a 2× gain probe was used, together with an IDAC-4 interface board and Autospike32 software

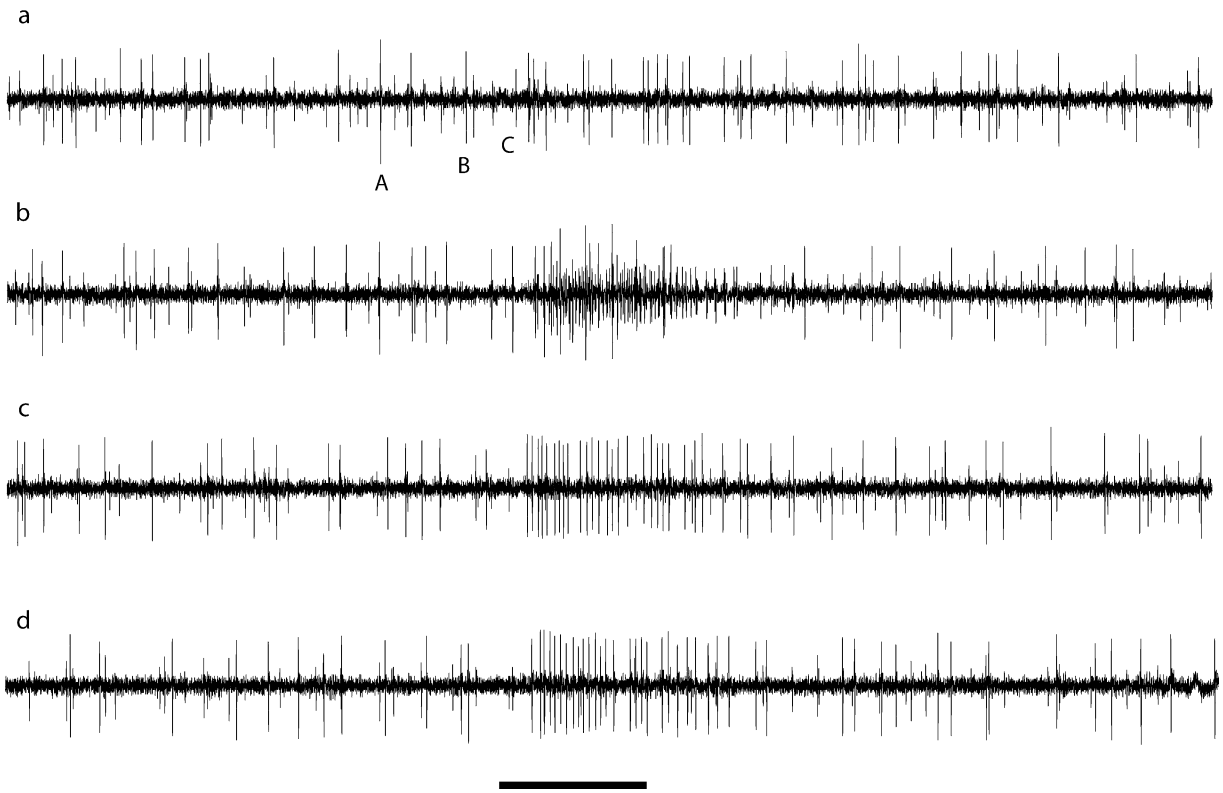

Figure 1 Responses of a female S4 sensillum to **(a)** blank (hexane), **(b)** *Daucus carota* extract, **(c)** *Picea abies* extract, and **(d)** male *Trioza apicalis* extract. Three ORNs give rise to 3 spike amplitudes (A, B, C). The black bar indicates the 0.5-s stimulation.

(all from Syntech, Hilversum, The Netherlands). The indifferent electrode was inserted into the right eye, and the recording electrode probed the 4 sensillar cavities on the left antenna. Henceforth, the sensilla investigated here will be referred to as S1 through S4, named for convenience according to their relative positions on the antenna (Kristoffersen et al. 2006). The most proximal sensillum is S1 and the most distal is S4.

A charcoal purified and humidified airflow of 5 ml/s was blown over the antenna via silicone and glass tubing (5.9 mm internal diameter [i.d.]) with the glass tube opening situated approximately 10 mm from the antenna. A loaded stimulus pipette was attached to a silicone tube connected to a stimulus puffer device (Syntech), the tip of the pipette inserted into a small hole in the glass tubing aiming toward the antenna, and a 0.5-s puff of the stimulus was introduced into the airflow and blown over the antenna. Fresh stimulus pipettes were prepared every day. Responses were calculated as the total number of action potentials (spikes) during 1 s after onset of stimulation, minus the number of spikes during 1 s immediately before onset of stimulus (spikes per second). To achieve net responses, we subtracted the value of the response elicited by the blank.

To identify the active components within the extracts eliciting responses in SSRs, we proceeded to perform GC-SSRs. We used a Hewlett Packard 5890 Series II plus gas chromatograph with a nonpolar HP1 column (30 m × 0.25 mm i.d.) and hydrogen as carrier gas. The temperature at injection was 60 °C and raised by 10 °C/min, and final temperature was 250 °C. By means of a split cross at the end of the column, half of the injected sample (2–4 µl) was led to the flame ion detector and the other half through a heated transfer line out over the antenna.

Chemical identification

Electrophysiologically active components of the extracts were identified by means of GC-MS. We used a Hewlett Packard 5890 Series II GC equipped with a nonpolar HP1 column and helium as carrier gas, coupled with a HP 5972 mass selective detector. Retention times and mass spectra of active peaks in the extracts were compared with mass spectra from MS libraries (Wiley257 and NBS75K) and candidate compounds identified by means of diagnostic peaks in the mass spectrum. The identity of some compounds was confirmed by comparison with mass spectra and retention times of synthetic reference samples. Active compounds identified and confirmed at an early stage of the experiments were used in dose–response experiments to determine the sensitivity and identity of the responding ORNs.

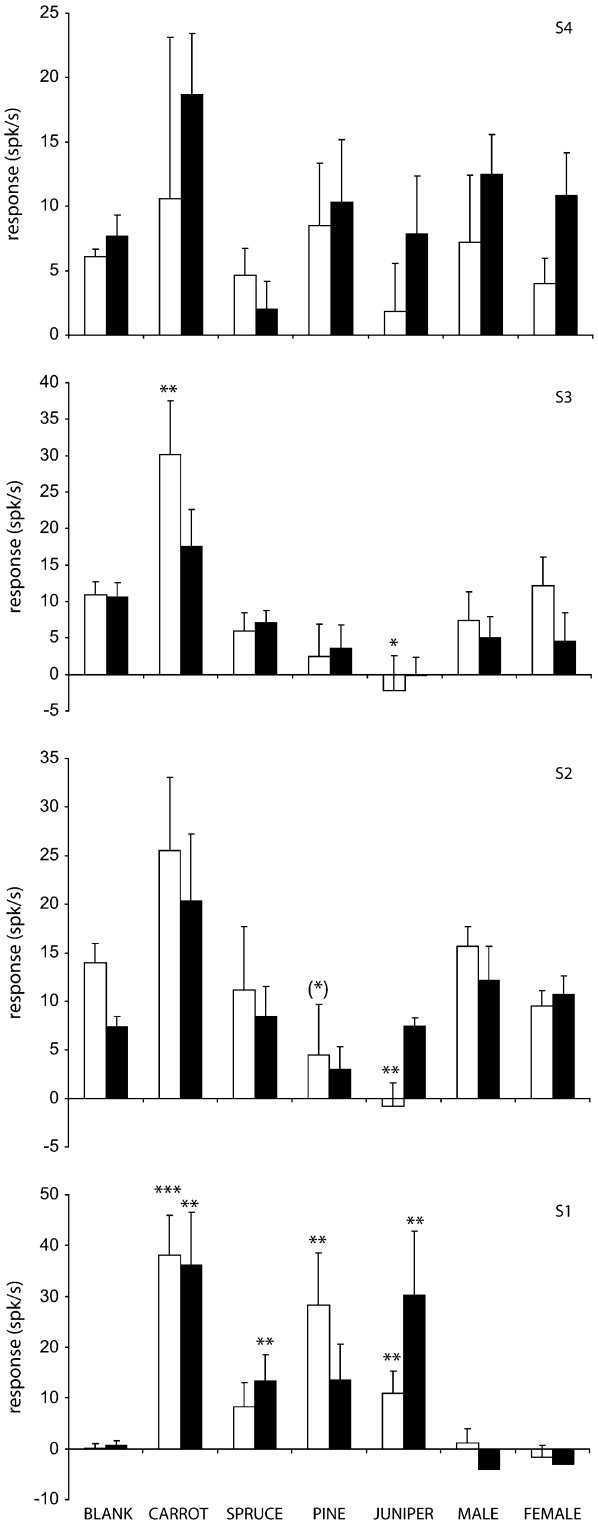

Figure 2 Responses from the 4 sensillar positions (S1–S4) to various host plant extracts and extracts of male and female *Trioza apicalis*. White bars represent males and black bars females. Asterisks indicate responses that significantly differ from the corresponding blank response (Mann–Whitney *U*-tests, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). The *n*-values range from 5 to 14, except for S1 responses to insect extracts in females, where $n = 2$ and no statistics have been performed. Error bars show standard error.

Statistics

Responses to extracts were compared with corresponding blank responses and statistically evaluated with Mann–Whitney *U*-tests. Dose–response series were tested with analysis of variances (ANOVAs) and post hoc Tukey's tests. Log-transformed values were used where necessary. All statistical analyses were performed in SPSS 11.0 for Windows.

Results

Responses to extracts

All sensilla contained at least one neuron responding to our positive control stimulus (carrot) and one or several of the extracts. Up to 3 spike amplitudes were observed, corresponding to the 3 ORNs found within a sensillum (Kristoffersen et al. 2006). We denoted the neurons according to spike amplitude (A = large, B = intermediate, and C = small) (Figure 1). In many recordings, most individual spikes from different neurons could not be reliably separated although overall amplitude differences between neurons were generally distinguishable. We therefore grouped responses from all 3 neurons when quantifying response strength to extracts and synthetic compounds.

All 4 sensilla responded to some or all the extracts tested but few were statistically different from the corresponding blank response (Figure 2). Overall, carrot odor was the most effective stimulus, eliciting the strongest responses. S2 and S3 generally gave higher blank responses than S1 and S4. The response pattern for S1 stands out from the other 3 sensilla, in displaying very low responses to the blank and *Trioza* extracts and in displaying significant responses to conifer extracts, in particular pine (*P. sylvestris*) and juniper (*J. communis*). When comparing the sexes, male S2 displayed a greater blank response than the female (Mann–Whitney *U*-test, $Z = -2.34$, $P < 0.05$). Juniper extract gave a significantly lower spike frequency than the blank in male S2 but not in female S2 (Mann–Whitney *U*-test, $Z = -2.21$, $P < 0.05$). There was a trend toward male S3 responding more strongly to carrot extract than female S3 (Mann–Whitney *U*-test, $Z = -1.94$, $P = 0.053$). There were no further differences between the sexes (Mann–Whitney *U*-tests, not shown).

The GC-SSR

In order to identify electrophysiologically active components of the complex extracts, we performed a total of 53 completed GC-SSRs, and 41 of these revealed responses to a variety of peaks in all types of extracts tested (Table 1). Although many potential responses of varying quality were recorded, it was not always possible to differentiate responses from artifacts or random fluctuations of the spike frequency without replicates. Hence, we selected only the 9 peaks that had elicited responses in at least 2 GC-SSR runs for further analyses, as a compromise between reliability and what could be obtained in reality. In many GC-SSRs, we

Table 1 The number of GC-SSR runs featuring any responses over the total number of runs performed for each sensillum position and extract type

Extract	S1		S2		S3		S4	
	♂	♀	♂	♀	♂	♀	♂	♀
Daucus carota	2/4	1/1	3/3	4/4	3/3	5/5	1/1	1/2
Picea abies	1/2		5/5					1/1
Pinus sylvestris	1/1		2/2	1/1				
Juniperus communis	2/2	2/2						1/1
Trioza apicalis ♂♀	0/1			1/3	0/2	0/1		
T. apicalis ♂				0/1		1/1		
T. apicalis ♀				1/1		0/1	1/1	

Out of 53 successfully completed runs, 41 yielded responses to one or several peaks.

were able to distinguish, based on spike amplitudes, which of the 3 neurons in a sensillum that was activated by a specific compound.

The identity of 4 individual compounds—nonanal, terpinolene, terpinene-4-ol, and (Z)-3-hexenal—could be confirmed by GC-MS and subsequent comparison with synthetic references, whereas 2 additional terpenes were tentatively identified based on comparisons with mass spectral databases (Table 2). All 4 compounds whose identities were confirmed were active components in *P. sylvestris* extracts and all but terpinolene in *P. abies* extracts. At least 4 compounds in the *D. carota* extract were active, but compound 7 in particular gave repeatable and distinct responses (Table 2, Figure 3A, Figure 4). Nonanal was an active compound in *T. apicalis* mixed and male extracts (Figure 3B). Interestingly, nonanal also occurred as an active component in *P. abies* and *P. sylvestris* extracts (Table 2). Terpinene-4-ol was active in all 3 kinds of conifer extracts (Table 2) and was one of 5 peaks in *P. sylvestris* extracts that gave repeated responses (Figure 3C). Terpinolene was found only in *P. sylvestris* extracts (Figure 3C).

Dose–response experiments

Dose–response experiments with the 4 identified substances overall showed similar patterns for both sexes (Figure 5). Results for each sex, sensillum, and stimulus were tested with ANOVAs and post hoc Tukey's tests (Appendix). There were substantial differences in variance between series, which causes the discrepancies between significance levels within sensilla. The highest dose of nonanal was active in S4 in both sexes, and also in S2 and S3, although much weaker in males than in females. S1 respond neither to nonanal nor to (Z)-3-hexenal. Instead, S1 in both sexes was sensitive to the conifer odors terpinolene and, especially, terpinene-4-ol. Terpinolene was not significantly active in S2, S3, or S4. Terpinene-4-ol showed dose–response trends in all sensillar positions, but sensitivity for this compound was highest in the S1 sensillum. Significant responses to (Z)-3-hexenal were shown in S2 and S3, stronger in females.

Characterization of neuron classes and grouping of neurons in sensilla

Based on responses to one or more active components of the extracts, we characterized 4 suggested classes of ORNs (W, X, Y, Z). Some responses could not be assigned to any specific class of receptor neuron and were grouped as "undetermined." A compilation of the active peaks, along with the suggested classes of ORNs, is given in Table 2.

Based on responses from both GC-SSRs and dose–response trials with synthetic compounds, we give a tentative representation of how different classes of neurons are clustered in the sensilla S1–S4 (Figure 6). As mentioned above, positions A–C represent different relative spike amplitudes, although these differences were sometimes hard to distinguish and not always consistent between recordings. It appears that all 4 sensilla contain one neuron of class X, responding primarily to the carrot-specific compound 7, although we did not demonstrate its presence in female S4 sensilla in GC-SSR (Table 2). Nevertheless, responses to carrot extract in SSRs from this position were not qualitatively different from the others (Figures 1 and 2), which supports this classification. These neurons consistently displayed the smallest spike amplitudes in all recordings. Otherwise, the sensilla appear to be of 2 main types. S1 represents a unique type of sensillum containing the 2-neuron classes W and Z, which were not found in the other sensilla, whereas S2, S3, and S4 all contain a neuron of class Y. This difference is consistent with sensilla S2–S4 responding to nonanal, whereas S1 does not. We did not find any compound eliciting responses from the A cell in sensilla S2–S4, meaning that these sensilla could possibly be further divided into subtypes. Our characterization of individual cell types does not indicate any sexual dimorphism in the S1–S4 sensilla.

Discussion

Electrophysiological studies of insect olfaction have traditionally focused on few insect groups, primarily Lepidoptera, although the fruit fly *Drosophila* has been instrumental in several breakthroughs during the last few years (see references in Jaquin-Joly and Merlin 2004; Vosshall and Stocker 2007). Many other insects still remain rather novel territory for this approach, and among these are the Homoptera. Within the Homoptera, the Aphidoidea is the most studied group. Dawson, Griffiths, Janes, et al. (1987) and Dawson, Griffiths, Pickett, et al. (1987) deployed the GC-SSR technique on aphids and identified a sex pheromone as well as plant compounds synergizing an alarm pheromone in 2 aphid species. GC-SSR is a particularly suitable technique when the number of responding receptors is low (Wadhams 1982; Van Der Pers and Löfstedt 1983; Wibe 2004). More recently, electrosensillogram recordings have

Table 2 A compilation of active peaks in GC-SSRs, sorted according to retention times

Peak	Extract	*n*	Responding positions	Suggested responding neuron class					Identified as
				W	X	Y	Z	?	
1	*Picea abies, Pinus sylvestris*	8	MS2, FS4			×			(Z)-3-hexenal[a]
2	*Daucus carota*	2	FS1, FS2		×			×	β-Phellandrene[b]
3	*P. sylvestris*	3	MS1, MS2		×				Terpinolene[a]
4	*P. abies, P. sylvestris, Trioza apicalis* ♂♀, *T. apicalis* ♂	5	MS2, FS3 FS4			×			Nonanal[a]
5	*Juniperus communis, P. sylvestris*	3	FMSI	×					Unidentified
6	*J. communis, P. abies, P. sylvestris*	6	FMSI				×		Terpinene-4-ol[a]
7	*D. Carota*	14	FSI, FMS2, FMS3, MS4		×				Unidentified sesquiterpene
8	*D. Carota*	2	FS1, MS2					×	Unidentified
9	*D. Carota*	3	FMS1				×		(Z)-α-bisabolene[b]

The responding sensillar positions and ORNs are given for each compound, along with a tentative classification of the responding neuron classes (W–Z, ? = undetermined). *n*, response replicates; F, female; M, male; S, sensillum.
[a]Identity determined by GC-MS, followed by coinjection with synthetic standards.
[b]Identification by comparisons with mass spectra from reference database.

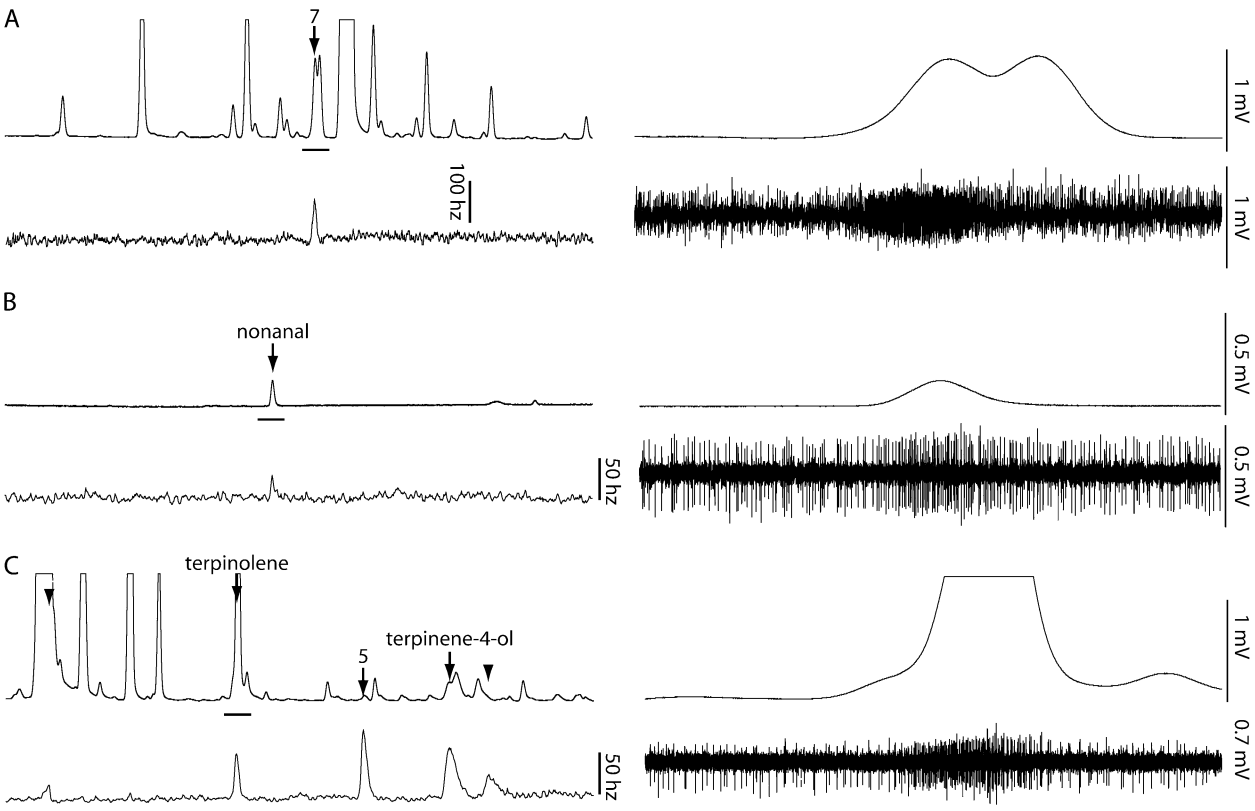

Figure 3 Examples of responses in GC-SSRs. On the left are 200 s excerpts. The black horizontal bars mark the 10-s response sequences shown to the right. On top are GC traces, below the sensillar responses. The designation of indicated peaks refer to Table 2. **(A)** Female S3 sensillum responding to peak 7 in *Daucus carota* extract (Figure 4). **(B)** Female S3 responding to nonanal in a *Trioza apicalis* male extract. **(C)** Male S1 responding to terpinolene, peak 5, and terpinene-4-ol in *P. sylvestris* extract. Two more responses without associations to visible peaks are indicated (arrowheads only), but because these responses could not be replicated, they are included neither in our analyses nor in Table 2. Shown on the right is the response to terpinolene.

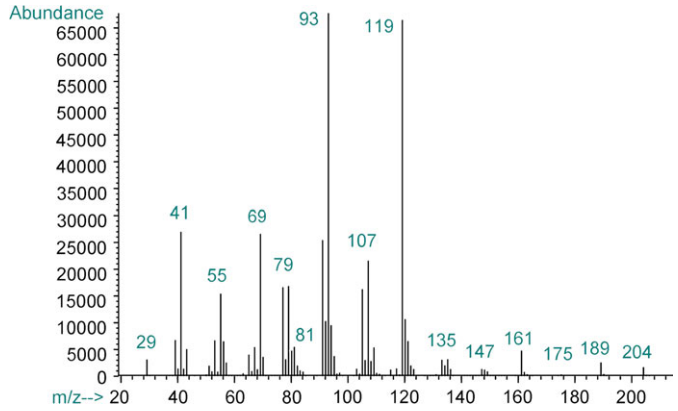

Figure 4 The mass spectrum of the unidentified peak 7, which elicited the highest number of responses of all components in the extracts. The compound is a sesquiterpene with a mass spectrum similar to a bergamotene.

been used for characterizing different olfactory sensilla in *Aphis fabae* (Park and Hardie 2004). Concerning Psylloidea, there is only one electrophysiological study published to date, in which Soroker et al. (2004) were able to demonstrate electroantennogram (EAG) responses in *Cacopsylla bidens* to headspace volatiles from infested and uninfested host plants.

We have recorded repeatable responses to various bouquets and single odorants from all S1–S4 sensilla, thereby confirming the olfactory function of this sensillum type. We can conclude that the 3 ORNs present in a single sensillum have different response spectra. The data presented here are ascribed to the larger of the 2 sensilla located within the 4 sensillar cavities. Although highly unlikely due to its hidden position, we cannot altogether exclude the possibility that we at times may have made contact also with the small sensillum positioned underneath. The modality of this sensillum is uncertain, and it is innervated by one ORN (Kristoffersen et al. 2006). The setup of the S1–S4 sensilla in total allows for a maximum of 12 unique ORNs. We have established that the total number of neuron classes occurring in these sensilla is in fact considerably fewer than 12. With a few neurons remaining to be characterized, we have suggested 4 functionally different classes, meaning that the total number of ORN classes in the *T. apicalis* system will be decidedly lower than 50. If the remainder of its olfactory system displays a similar degree of redundancy, it is the most limited olfactory system characterized in adult neopteran insects.

The limited number of components in its peripheral olfactory system means that *T. apicalis* presumably faces a severe conflict between scope and sensitivity. A low number of ORN classes probably limits the receptive range and discriminatory capacity of the olfactory system. Our recordings do not suggest that ORNs are unusually broadly tuned to compensate for this. On the contrary, each neuron responds to very few compounds among the complex extracts, even if we include the few spurious responses that were excluded from our detailed analysis. Nevertheless, *T. apicalis* sensilla display considerable redundancy, with as much as 4 out of 12 neurons belonging to the ORN type X. High redundancy increases sensitivity to important key volatiles by improving the signal-to-noise ratio in the system (Larsson and Svensson 2005). Increased investments in selected types of ORNs to improve sensitivity to compounds of special significance are found not only among sex pheromone detection systems but also at a smaller scale, for example, in host detection systems of *Drosophila* species (Dekker et al. 2006).

For a specialist insect such as *T. apicalis*, with a very small olfactory system, utilizing chemically similar plants is likely to be adaptive, allowing for further specialization and redundancy. In a study by Valterová et al. (1997), a multivariate analysis of the relative amounts of monoterpene content in *D. carota* ssp. *sativus*, *D. carota* ssp. *carota* (wild carrot), 13 other species of Apiaceae and 2 of the carrot psyllid winter shelter plants, *P. abies* and *J. communis*, revealed a striking chemical similarity between the host and over-wintering plants of *T. apicalis*. *Dacus carota*, *P. abies*, and *J. communis* stood out from the other, nonhost Apiaceae species (Valterová et al. 1997). This means that the overlap of the chemical profiles of the plant material tested here is extensive. Some of the electrophysiologically active compounds characterized here (β-phellandrene, terpinolene) are also the same as found by Valterová et al. (1997), but we note that none of the 4 compounds conclusively identified in this study has been suggested before in relation to *Trioza* or similar insects. Four of the 9 active compounds discussed here were found in more than one type of extract ((Z)-3-hexenal, nonanal, peak 5, terpinene-4-ol), and comparisons with Valterová et al. (1997) suggest that the degree of overlap should have been even higher. This even included the *Trioza* extracts in the case of nonanal, which was an active component in *P. abiae* and *P. sylvestris* extracts as well. All 4 sensilla responded to carrot odors, and these responses were often the strongest. The most consistent response in GC-SSR experiments was to the carrot-specific compound 7. Compound 7 is a sesquiterpene with a mass spectrum very similar to that of bergamotene, but its identity still remains to be determined. Whereas none of the neurons characterized here responded exclusively to either carrot or conifer volatiles, neurons of type X nevertheless appear to be primarily tuned to the unidentified carrot compound 7, indicating that some neuron types could selectively mediate migration in either direction.

Our data show that S1 is more sensitive to conifer extracts than the other 3 sensilla. Also, S1 never responded to insect extracts or the active component therein, nonanal, leading us to conclude that this sensillum lacks the particular cell type sensitive to this compound. Pheromones have not been identified in psylloids, although there have been indications of pheromonal activity in behavioral and electrophysiological experiments (Soroker et al. 2004). The EAG study on the pear psylla *C. bidens* showed differences in the responses from males and females. Male

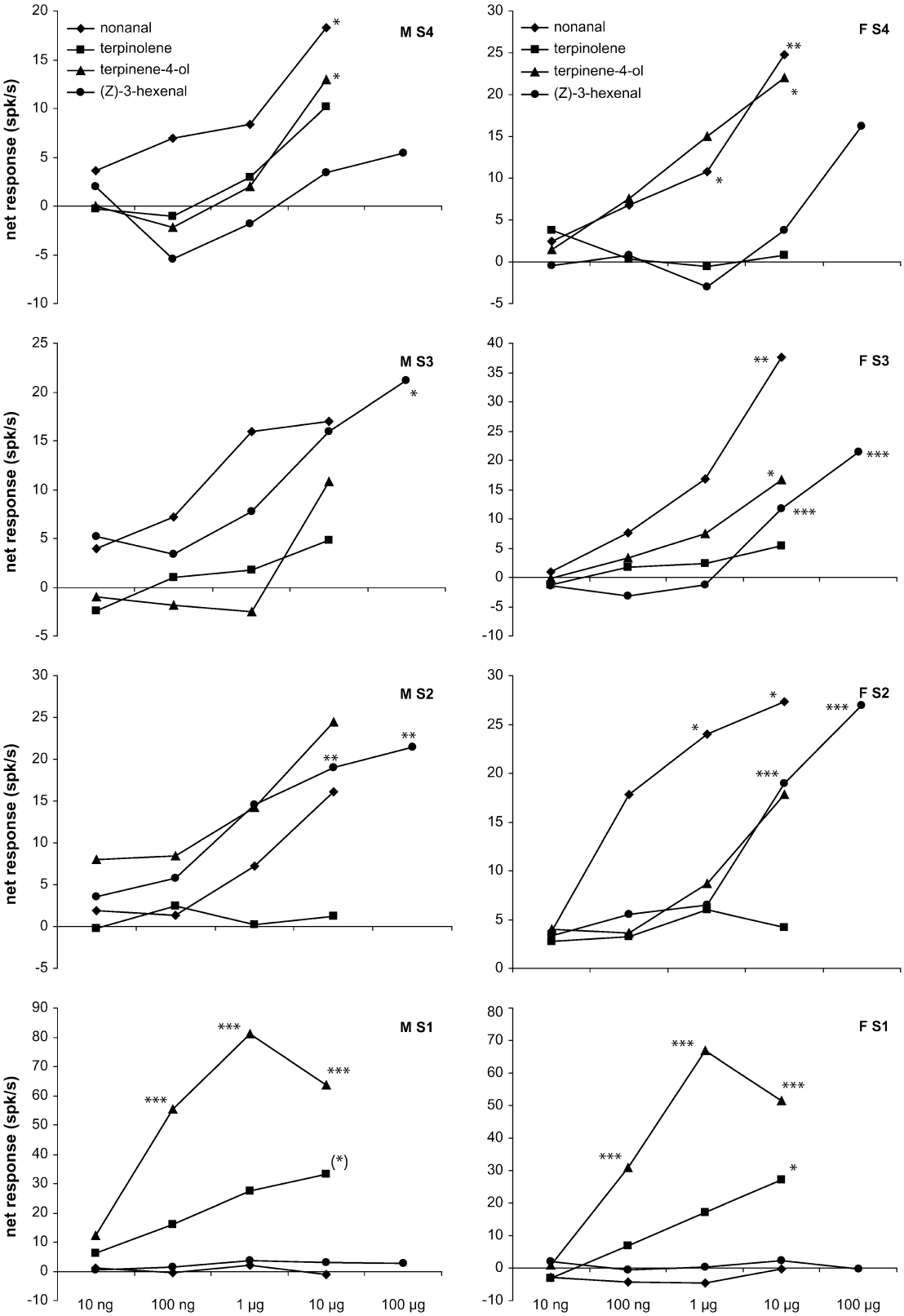

Figure 5 Dose–response curves for 4 single compounds identified via GC-SSR and GC-MS, nonanal, terpinolene, terpinene-4-ol, and (Z)-3-hexenal. Stars indicate responses significantly different from the blank when tested with ANOVAs and Tukey's post hoc tests (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Plotted here are net responses. n = 5 or 6 in all cases. M = male, F = female, S1–S4 = sensillar positions. See Appendix for F- and P-values.

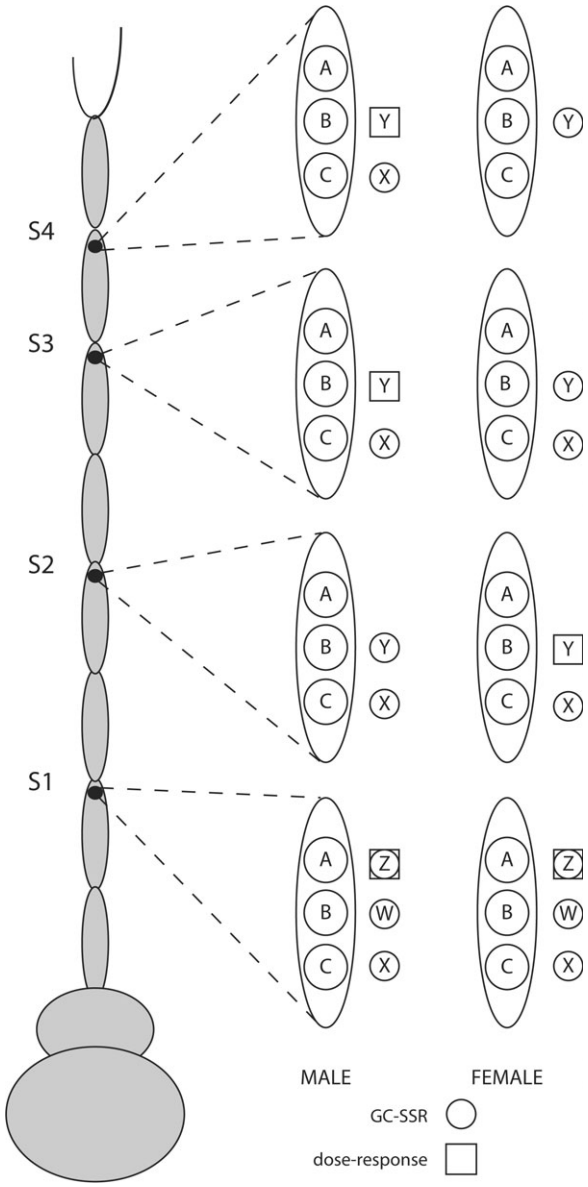

Figure 6 A schematic illustration of a *Trioza apicalis* antenna, showing the positions on the flagellum of the 4 olfactory sensilla studied here (S1–S4). Each sensillum is innervated by 3 ORNs, generating 3 spike amplitudes (A, B, C). Responses to single compounds in GC-SSR and dose–response experiments reveal a redundancy of cell types within these sensilla, and based on our data, we propose 4 distinct neuron classes (W, X, Y, Z), arranged in a specific pattern in the 4 sensilla (cf., Table 2, Figure 5).

antennae responded only to odors from female-infested hosts. The authors suggested that females emit a sex pheromone, attracting males, although there was no sexual dimorphism in the antennae revealed by scanning electron microscopy (Soroker et al. 2004). Species utilizing sex pheromones usually show considerable sexual dimorphism in antennal morphology (Keil 1999). Here, we have identified nonanal as a compound present in extracts from insects and plants that elicits repeatable dose–response patterns from 3 of the sensilla investigated here. Nonanal was found in male and mixed extracts but not pure female extracts. Both sexes responded to it electrophysiologically, rather suggesting that this could be an aggregation or alarm substance or just simply an occasional by-product of ingested host material. The function and significance of nonanal and any synergistic effects between host odors and potential pheromones also remain to be investigated. The current study revealed only very small physiological differences between the sexes. Morphologically, male and female antennae are identical in their sensillar setup (Kristoffersen et al. 2006). An accompanying study of *T. apicalis* and other hemipteran insects has shown that the antennal lobes of psyllids and aphids lack glomeruli. Morphological studies of the antennal lobes would thus not reveal macroglomeruli or other antennal lobe structures that are often associated with pheromone communication in insects (Kristoffersen et al. 2008).

By extending the GC-SSR studies initiated here, a wider range of potential kairomones and pheromones will surely be identified. Considering the low total number of ORNs in the antenna, even a complete functional map of all sensilla and ORNs is likely within reach. The tiny but highly specialized olfactory systems of psyllids could thus serve as interesting models to study olfaction in an evolutionary context, including functional adaptations of olfactory systems to their specific odor environments.

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Appendix

Results of ANOVAs and post hoc Tukey's tests for dose responses (Figure 5)

Sensillum	Stimulus	*F*	Degrees of freedom	*P*	Tukey's post hoc tests
M S4	Nonanal	2.67	4	0.056	Dose 4: $P < 0.05$
	Terpinolene	—	—	ns	—
	Terpinene-4-ol	4.01	4	<0.05	Dose 4: $P < 0.05$
	(Z)-3-hexenal	—	—	ns	—

Appendix Continued

Sensillum	Stimulus	*F*	Degrees of freedom	*P*	Tukey's post hoc tests
M S3	Nonanal	2.67	4	0.055	—
	Terpinolene	—	—	ns	—
	Terpinene-4-ol	—	—	ns	—
	(Z)-3-hexenal	3.03	5	<0.05[a]	Dose 5: $P < 0.05$
M S2	Nonanal	—	—	ns	—
	Terpinolene	—	—	ns	—
	Terpinene-4-ol	—	—	ns	—
	(Z)-3-hexenal	6.85	5	<0.001	Dose 4 and 5: $P < 0.01$
M S1	Nonanal	—	—	ns	—
	Terpinolene	3.00	4	<0.05	Dose 4: $P = 0.051$
	Terpinene-4-ol	30.97	4	<0.001	Dose 2, 3, and 4: $P < 0.001$
	(Z)-3-hexenal	—	—	ns	—
F S4	Nonanal	15.14	4	<0.001	Dose 3: $P < 0.05$, dose 4: $P < 0.001$
	Terpinolene	—	—	ns	—
	Terpinene-4-ol	3.58	4	<0.05	Dose 4: $P < 0.05$
	(Z)-3-hexenal	—	—	ns	—
F S3	Nonanal	6.24	4	<0.01	Dose 4: $P < 0.01$
	Terpinolene	—	—	ns	—
	Terpinene-4-ol	3.34	4	<0.05	Dose 4: $P < 0.05$
	(Z)-3-hexenal	16.57	5	<0.001	Dose 4 and 5: $P < 0.001$
F S2	Nonanal	4.73	4	<0.01[a]	Dose 3: $P < 0.05$, dose 4: $P < 0.05$
	Terpinolene	—	—	ns	—
	Terpinene-4-ol	—	—	ns	—
	(Z)-3-hexenal	7.16	5	<0.001	Dose 4 and 5: $P < 0.001$
F S1	Nonanal	—	—	ns	—
	Terpinolene	3.52	4	<0.05	Dose 4: $P < 0.05$
	Terpinene-4-ol	25.56	4	<0.001[a]	Dose 2, 3, and 4: $P < 0.001$
	(Z)-3-hexenal	—	—	ns	—

Where required, data were log transformed before analysis.
[a]Analysis was performed on log-transformed data.

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