1. Introduction

The genus *Eucalyptus* contains some of the most commonly cultivated hardwood timber species around the world (Santos et al., 2004). In addition to timber production, *Eucalyptus* trees are used for medicine, ornamental foliage, as landscape trees or wind breaks (Doughty, 2000). In the late 1990s, the commercial usage of *Eucalyptus* spp. increased dramatically in range and distribution across Europe, North and South America (Hodkinson, 1999). However, over the past three decades *Eucalyptus* growing as exotics outside Australia have suffered increased biotic stress caused by a number of Australian and endemic herbivorous insects (Gill et al., 1998; Paine et al., 2011). Among these pests is the small (1.5–2.0 mm) blue gum psyllid, *Ctenarytaina eucalypti* (Maskell) (Sternorrhyncha: Psyllidae), which feeds preferentially on waxy juvenile *Eucalyptus globulus* foliage (Burckhardt et al., 1999; Purvis et al., 2002; Hollis, 2004). Extralimitally, this psyllid has been reported attacking more than 25 eucalypts, including *Eucalyptus dunnii*, *Eucalyptus gunnii*, *Eucalyptus cordata* (all subgenus *Symphyomyrtus*) and even *Corymbia citriodora* (Burckhardt et al., 1999; Dahlsten et al., 1998a; Hodkinson, 1999; Purvis et al., 2002; Santana, 2005; Santana and Burckhardt, 2007). *C. eucalypti* is native to Australia but has been introduced into at least 18 countries across the world (Burckhardt, 1998; Burckhardt et al., 1999; Costanzi et al., 2003; Hodkinson, 2007; Olivares, 2000; Purvis et al., 2002; Taylor, 1997).

Female blue gum psyllids can lay 20–100 eggs, normally as a group in the leaf buds and the axils of young leaves of young plants (Cadahia, 1980; Santana and Burckhardt, 2007). The psyllid can produce up to four generations per year, depending on climate and plant suitability. The larval instars and the adults feed on plant sap. While feeding they excrete large amounts of honeydew and waxy secretions that act as a growth medium for sooty molds (Dahlsten et al., 1998b; Santana and Burckhardt, 2007). According to Santana and Burckhardt (2007), damage by *C. eucalypti* can be induced directly by sucking the sap, or indirectly by injecting toxic substances from the saliva. The direct effects of feeding include
2. Materials and methods

2.1. Insects

Psyllids collected from *E. globulus* growing at Belfield (Dublin, Ireland) and a plantation of *E. globulus* near Clonbinane (Victoria, Australia) were shipped to Sweden and thereafter cultured in a climate chamber. Live psyllids were sent from Melbourne under Australian Government export permit (WT2012-764) by MNX-GlobalLifeSciences. Psyllids were reared on *E. globulus* seedlings under 20:4 light:dark cycle, 20:15 °C (light:dark) temperature cycle and 50–60% RH. Adults of both sexes were used for SSR. Sexes were separated by the presence of proctiger (♂) or terminalia (♀).

2.2. Headspace volatile collection and chemical analyses

*E. cordata* seedlings were used in headspace volatile collection. Volatile collection was performed for 6 h between 12:00 and 18:00 under 4 h light and 2 h dark at room temperature. The plants were separated by the presence of proctiger (♂) or terminalia (♀).

The collection was eluted using 250 μl redistilled hexane (purity >98%, LiChrosolv®) and condensed to 30 μl. The samples (2 μl injected volume) were then injected into the GC–MS (gas chromatography–mass spectrometry) equipped with a 7683 injector, 5973 MSD detector and splitless injection system. The GC had a 30 m Innowax capillary column (Agilent 19091N-033 MS, 0.25 mm i.d., 0.25 μm film), using helium as carrier gas at 6.45 psi (Agilent Technologies, Santa Clara, USA). The oven temperature was programmed as follows: 30 °C (held for 2 min) and then increased at a rate of 10 °C/min up to 220 °C (held for 10 min). The inlet temperature was 225 °C and the interface temperature was 280 °C. Identification of compounds was done based on comparison of mass spectra with those of ADAMS Library and synthetic reference samples.

2.3. Single sensillum recordings

A live *C. eucalypti* was placed ventrally in a 100 μl disposable plastic micropipette tip, with its head and antennae protruding. The pipette tip was placed in dental wax on a glass slide and the antennae fixed using double sided sticky tape on a cover slip. By using thin copper wire, the position of the antennae was adjusted to a convenient angle. For closer view of the antennae and sensillar cavities, a microscope (Nikon eclipse E600FN: 750 × magnification) was used during recordings.

Tungsten microelectrodes were sharpened using saturated KNO₃ solution and 20 V AC (Power Supply Unit MA4852). The reference electrode was inserted into the head of the insect, normally through the eye, and the recording electrode in one of the sensilla, aided with a Piezo micromanipulator (PM 10, Mätzler-Steindorf, Germany). The recording electrode was connected to an universal AC/DC amplifier probe (gain 10 ×) that, in turn, was connected to an IDAC-4 interface board (all from Syntech, Kirchzarten, Germany). Autospike software v3.9 (Syntech) was used to record the response. Charcoal-purified and humidified airflow at 1.81/ min (controlled by airflow meter, Porter Instruments, USA) was continuously blown over the antenna via silicone and glass tubing (5.9 mm i.d.). The outlet of the tube was placed approximately 25 mm from the antenna.

The odorant test panel comprised 39 synthetic compounds identified in the present or previous studies from the headspace of various *C. eucalypti* hosts (Table 1). In addition, a crushed *E. cordata* leaf was also tested to check whether *Eucalyptus* contains physiologically active compounds that were lacking in our panel of synthetic test odors. The compounds were diluted in paraffin oil (except for β-ocimene that was diluted in hexane) and applied (10 μl) on 2 × 1 cm filter paper strips (No. 3, Whatman, Maidstone, United Kingdom) inside glass Pasteur pipettes (150 mm soda lime glass, VWR International, Stockholm, Sweden). Response specificity of OSNs was characterized using a screening dose of 10 μg compound on the filter paper. Compounds were tested in random order. Dose–response tests were performed on additional sensilla, using doses from 1 ng to 10 μg (lowest dose tested first). In these recordings, we focused on the compounds (i.e. linalool, β-caryophyllene, 1-hexanol, 23-hexenol, Z-3-hexenyl acetate, and E2-hexenal) that elicited the strongest OSN responses at the 10 μg screening dose, since responses to weakly activating compounds are normally absent when the dose is lowered (e.g. Andersson et al., 2009; Larsson et al., 2001). During stimulation, headspace from the stimulus pipettes was introduced into the continuous airflow, and hence to the insect, at a rate of 0.2 l/min for 0.5 s (controlled by stimulus controller CS-02, Syntech). No pulse compensatory flow was used during puffing. Since repeated puffing of stimulus pipettes significantly reduces the airborne stimulus concentration (Andersson et al., 2012b), pipettes were reloaded.
after a maximum of 10 puffs during screening or after two puffs in dose–response tests.

The OSNs in one of the sensilla responded the strongest to two compounds (β-caryophyllene and linalool activating two different OSNs) with vastly different evaporation rates from paraffin oil (ca. 30 times lower evaporation of β-caryophyllene; E. Hatano, personal communication). Thus, to provide more accurate sensitivity estimates for these two neurons, GC-coupled SSR was performed by injecting 10 ng of each compound diluted in hexane (for details see Kristoffersen et al., 2008). GC–SSR was not used for the other strongly activating synthetic compounds, 1-hexanol, Z3-hexenol, and Z3-hexenyl acetate, since the evaporation rate of different green leaf volatiles (GLVs) from paraffin oil is similar (Andersson et al., 2012b).

### 2.4. Data analysis

OSN responses were analyzed offline using Autospike v3.9 (SynTech). Net odor responses were calculated by counting the number of spikes during the first 0.5 s of the response and subtracting the number of spikes in the blank (5°C) during screening were categorized.

<table>
<thead>
<tr>
<th>Chemicala</th>
<th>Chemical source</th>
<th>Purity (%)</th>
<th>Chemical class</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>Fluka</td>
<td>99</td>
<td>MT</td>
<td>E. dunnii, E. cordata, C. citriodora, E. globulus</td>
<td>This study, Street et al. (1997), Zini et al. (2001, 2002) and Barata et al. (2000, 2002)</td>
</tr>
<tr>
<td>(−)-β-Pinene</td>
<td>Fluka</td>
<td>&gt;99</td>
<td>MT</td>
<td>E. dunnii, E. cordata, C. citriodora, E. globulus</td>
<td>This study, Wirthensohn et al. (2000), Zini et al. (2001) and Barata et al. (2000, 2002)</td>
</tr>
<tr>
<td>α-Phellandrene</td>
<td>Aldrich</td>
<td>&gt;95</td>
<td>MT</td>
<td>E. dunnii, E. cordata, E. globulus</td>
<td>This study, Zini et al. (2002) and Barata et al. (2000)</td>
</tr>
<tr>
<td>γ-Terpineone</td>
<td>G. Bergström</td>
<td>95</td>
<td>MT</td>
<td>E. dunnii, E. cordata, E. globulus</td>
<td>This study, Zini et al. (2002), Yassaa et al. (2000) and Barata et al. (2000)</td>
</tr>
<tr>
<td>Camphene</td>
<td>Aldrich</td>
<td>95</td>
<td>MT</td>
<td>E. saligna, E. globulus</td>
<td>Zini et al. (2002), Yassaa et al. (2000) and Barata et al. (2000)</td>
</tr>
<tr>
<td>(Z)-Fenchol</td>
<td>G. Bergström</td>
<td>&gt;99</td>
<td>MT–OH</td>
<td>E. globulus</td>
<td>Yassaa et al. (2000)</td>
</tr>
<tr>
<td>Limonene</td>
<td>Fluka</td>
<td>99</td>
<td>MT</td>
<td>E. dunnii, E. cordata, C. citriodora, E. globulus</td>
<td>This study, Street et al. (1997), Zini et al. (2002) and Barata et al. (2000, 2002)</td>
</tr>
<tr>
<td>(Z,E)-Linalool oxidec</td>
<td>Dragoco</td>
<td>92</td>
<td>MT</td>
<td>C. citriodora, E. globulus</td>
<td>Barata et al. (2000)</td>
</tr>
<tr>
<td>Tricyclene</td>
<td>Aldrich</td>
<td>95</td>
<td>MT</td>
<td>E. globulus</td>
<td>Yassaa et al. (2000)</td>
</tr>
<tr>
<td>Δ3-Carene</td>
<td>Aldrich</td>
<td>95</td>
<td>MT</td>
<td>E. globulus</td>
<td>Barata et al. (2000)</td>
</tr>
<tr>
<td>Δ2-Carene</td>
<td>G. Bergström</td>
<td>&gt;99</td>
<td>MT</td>
<td>E. cordata</td>
<td>-</td>
</tr>
<tr>
<td>P-Cymene</td>
<td>Acros</td>
<td>&gt;99</td>
<td>MT</td>
<td>E. cordata, E. globulus</td>
<td>This study, Barata et al. (2000)</td>
</tr>
<tr>
<td>Sabineone</td>
<td>Aldrich</td>
<td>99</td>
<td>MT</td>
<td>C. citriodora, E. globulus</td>
<td>Zini et al. (2001) and Barata et al. (2000)</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>Fluka</td>
<td>97</td>
<td>MT</td>
<td>E. cordata, E. globulus</td>
<td>This study, Barata et al. (2000)</td>
</tr>
<tr>
<td>α-Terpinenol</td>
<td>Aldrich</td>
<td>90</td>
<td>MT–OH</td>
<td>E. globulus</td>
<td>This study, Street et al. (1997) and Barata et al. (2000)</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>Fluka</td>
<td>&gt;99</td>
<td>MT–OH</td>
<td>E. cordata</td>
<td>-</td>
</tr>
<tr>
<td>Isopulegol</td>
<td>Sigma</td>
<td>&gt;99</td>
<td>MT–OH</td>
<td>C. citriodora</td>
<td>Zini et al. (2001)</td>
</tr>
<tr>
<td>(Z,E)-Thujone4</td>
<td>G. Bergström</td>
<td>96</td>
<td>MT=O</td>
<td>E. globulus</td>
<td>Barata et al. (2000)</td>
</tr>
<tr>
<td>Piperitone</td>
<td>G. Bergström</td>
<td>&gt;99</td>
<td>MT=O</td>
<td>E. globulus</td>
<td>Barata et al. (2000)</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>Aldrich</td>
<td>&gt;99</td>
<td>MT–acetal</td>
<td>E. dunnii, E. cordata, C. citriodora, E. globulus</td>
<td>This study, Street et al. (1997), Zini et al. (2001, 2002) and Barata et al. (2000, 2002)</td>
</tr>
<tr>
<td>α-Cubebene</td>
<td>G. Bergström</td>
<td>88</td>
<td>ST</td>
<td>E. globulus</td>
<td>Barata et al. (2000)</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>Fluka</td>
<td>98</td>
<td>ST</td>
<td>E. citriodora, E. globulus</td>
<td>Barata et al. (2000, 2002) and Lopes et al. (2002)</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>Fluka</td>
<td>99</td>
<td>C6–OH</td>
<td>E. globulus</td>
<td>Barata et al. (2000)</td>
</tr>
<tr>
<td>Z2-Hexenol</td>
<td>Aldrich</td>
<td>95</td>
<td>C6–OH</td>
<td>E. globulus</td>
<td>Barata et al. (2000, 2002) and Lopes et al. (2002)</td>
</tr>
<tr>
<td>Z3-Hexenol</td>
<td>Acros</td>
<td>98</td>
<td>C6–OH</td>
<td>E. cordata, E. globulus</td>
<td>Yassaa et al. (2000)</td>
</tr>
<tr>
<td>Z3-Hexenyl acetate</td>
<td>Sigma</td>
<td>99</td>
<td>C6–OAc</td>
<td>E. cordata, E. globulus</td>
<td>This study, Barata et al. (2000, 2002) and Lopes et al. (2002)</td>
</tr>
<tr>
<td>Ethyl-3- methylbutanoate</td>
<td>Aldrich</td>
<td>98</td>
<td>C7–OAc</td>
<td>E. globulus</td>
<td>Barata et al. (2002)</td>
</tr>
<tr>
<td>Citronellyl acetate</td>
<td>G. Bergström</td>
<td>96</td>
<td>C12–OAc</td>
<td>C. citriodora</td>
<td>Zini et al. (2001)</td>
</tr>
<tr>
<td>3-Hydroxy-2-butanone</td>
<td>Aldrich</td>
<td>&gt;97</td>
<td>C4=O</td>
<td>E. globulus</td>
<td>Barata et al. (2000, 2002) and Lopes et al. (2002)</td>
</tr>
<tr>
<td>E2-Hexenal</td>
<td>Aldrich</td>
<td>98</td>
<td>C8=O</td>
<td>E. cordata</td>
<td>-</td>
</tr>
<tr>
<td>6-Methyl-5-hepten-2-one</td>
<td>Sigma</td>
<td>99</td>
<td>C8=O</td>
<td>E. cordata</td>
<td>This study</td>
</tr>
<tr>
<td>(Z,E)-3,7-Dimethyl-2,6-octadienal</td>
<td>Aldrich</td>
<td>95</td>
<td>C10=O</td>
<td>C. citriodora</td>
<td>Zini et al. (2001, 2002)</td>
</tr>
<tr>
<td>Paraffin oil</td>
<td>Merck</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: HT: hemiterpene (5C), MT: monoterpane (10C), ST: sesquiterpene (15C),–OH: alcohol, =O: aldehyde and ketone, =OAc: acetate ester.

a Chiral compounds were tested as racemic mixtures unless otherwise indicated.
b Mixture of Z,E isomers:(30:70%).
c Mixture of Z,E isomers:(30:50%).
d Mixture of Z,E isomers:(83:17%).
e Gift from Prof. Gunnar Bergström.
based on number of spikes/s, where (0) <10 Hz, (+) = 10–29 Hz, (++) = 30–59 Hz, (+++) = 60–89 Hz, (++++) = 90–119 Hz, (+++++) = 120 Hz. We also analyzed temporal odor response profiles for strongly responding OSN types. This was done by counting spikes in 200 ms bins, starting at the onset of stimulation and ending 8 s after the onset. The average spontaneous activity during 2 s preceding the stimulation was subtracted from these responses to obtain the net responses. GC–SSR responses were quantified by counting the number of spikes during a 1 s period around the center of the compound peaks. Net responses were obtained by subtracting the spontaneous activity during the 1 s period just before the compound peaks.

3. Results

3.1. Headspace volatiles

Twelve volatile compounds were identified from the headspace collection of *E. cordata*. Additional compounds were present, but could not be identified (Supplementary material Fig. S1 and Table S1). The main compound that gives the characteristic odor of *Eucalyptus*, 1,8-cineole (eucalyptol), was present in the highest quantity followed by *P*-phellandrene and limonene. *P*-Pinene and *γ*-terpinene, as well as *P*-cymene were present in relatively high amounts. Some compounds that existed in low quantities were 6-methyl-5-hepten-2-one, the green leaf volatiles 3-hexenyl acetate, as well as the terpenes, terpinen-4-ol, β-pinene, and terpinolene.

3.2. Antennal morphology and general response characteristics

The antenna of the blue gum psyllid is filiform and contains four sensillar cavities, one on each of segment 4, 6, 8 and 9 (Fig. 1). We labeled the sensilla in the cavities: S1 (proximal), S2, S3 and S4 (distal) according to their location on segments 4–9. Recordings were obtained from all sensilla. In total, 42 adults (22 males, 20 females) were tested for responses to the synthetic compounds (see Table 2 and Fig. 3 for n-values for each sensillum in the screening and dose–response tests, respectively). In each sensillum, three OSNs were distinguished based on spike amplitudes (labeled A, B, and C neuron with large, intermediate, and small amplitude, respectively; Fig. 2A). However, the C neurons in S1–S4 never responded to any of the tested host volatiles. Inhibitory responses (decrease in spike activity) were elicited only by Z2-hexenol in S2, S3 and S4 (Fig. 2B).

Thirty-three of the 39 tested compounds elicited responses in at least one OSN type (Table 2). The remaining six compounds (linalool oxide, 3-carene, 2-carene, P-cymene, sabine, and piperitone) were sedentary (Table 2). 1,8-Cineole elicited only minor responses although this volatile dominates the headspace of *Eucalyptus* and is responsible for the characteristic (at least for humans) *Eucalyptus* smell. Response to blank stimuli (paraffin oil) was always below 10 Hz.

Males and females appeared to have the same or at least very similar classes of OSNs on their antennae. However, the strength of the responses and the presence of weak (+) responses varied somewhat between sexes for some of the compounds, especially in S2 and S3 (Table 2). The strongest OSN responses (in S1–S3) ranged between 120 and 160 Hz, and occasionally neurons fired at >190 Hz. These strong responses suggest that the key ligands for the receptors likely were included in the test panel.

3.3. OSNs in S1 responding primarily to β-caryophyllene and linalool

The strongest responses in the study were recorded from the B neuron in S1 that was highly specific for linalool (Table 2). Sometimes the spike activity was elevated for more than a minute after the onset of stimulation. In addition, the A neuron in S1 showed an intermediate (++) response to β-caryophyllene (total average for males and females combined: 47 Hz) and a weaker response to *(E/Z)*-β-ocimene (total average 27 Hz, weaker in males than in females). This OSN also showed a weak (+, male) or intermediate (+++, female) response to the crushed *E. cordata* leaf (Table 2). Both neurons were subjected to dose–response trials that indicated a response threshold for linalool below the 10 ng dose on the filter paper, whereas the A neuron was 10–100 times less sensitive to β-caryophyllene (Fig. 3A). However, since the evaporation rate of β-caryophyllene (sesquiterpene) from paraffin oil is ca. 30 times lower than that of linalool (monoterpene alcohol) (E. Hatano, personal communication), we also delivered the two compounds to the antenna by means of a GC (10 ng of each compound injected). The GC–SSR results indicated that when the two compounds are delivered at the same (presumably) airborne concentration, the responses in the two OSNs are more similar. However, the response in the B cell to linalool (73 spikes/s) was still stronger than the response in the A cell to β-caryophyllene (45 spikes/s) (Fig. 4; see Table 2 for comparison), β-Ocimene, the second most active compound in the A cell, was not tested through the GC since a previous study showed that the evaporation rates of other monoterpene hydrocarbons were similar to that of linalool (Andersson et al., 2012b). During the B cell response to linalool, the spontaneous activity of the A cell was simultaneously inhibited.

3.4. OSNs in S2 and S3 responding primarily to green leaf volatiles

The responses of both the A and B cell were similar in S2 and S3 (Table 2), especially in the B cell to the three most potent ligands. The B neurons in both sensilla responded most strongly to the GLVs 1-hexanol, Z3-hexenol and Z3-hexenyl acetate. The response to the individual GLVs in the B neuron varied from intermediate to very strong between contacted sensilla. In the S3 sensillum of three individuals there was no response at all to any of the GLVs. Since the OSNs within these sensilla might have been damaged or responsive only to compounds not present in the odor panel, they were not included in the calculation of average responses (Table 2). In both S2 and S3, some compounds (e.g. 3-methyl-1-butanol, Z2-hexenol, some ketones and acetate esters) that were inactive in males elicited weak (+) responses in females. The dose–response curves of the B neurons in S2 and S3 were highly similar and indicated that the response thresholds for Z3-hexenol, 1-hexanol and Z3-hexenyl acetate were near or below the 1 ng dose (Fig. 3B–C). Responses were slightly stronger to Z3-hexenol at all tested doses. The OSNs were less sensitive to E2-hexenal, with a response threshold between the 1 and 10 ng dose.

The A neurons in S2 and S3 displayed weaker responses to *(E/Z)*-β-ocimene and E2-hexenal (for details see Table 2). The other compounds in the test panel elicited only very weak responses in these sensilla. The crushed *E. cordata* leaf evoked stronger responses in the B neurons than in the A neurons.

3.5. OSNs in sensillum S4

In contrast to the other sensilla, OSNs within S4 did not respond strongly to any of the synthetic compounds or to the crushed leaf.
Fig. 2. Single sensillum recordings from *Ctenarytaina eucalypti*. (A) Three OSNs (A, B, C) could be distinguished based on differences in the size of the spike amplitude. (B) A weak excitation followed by inhibition (occasionally seen) in the B neuron of S2 and S3 in response to Z\(^2\)-hexenol (10 μg).
we do not draw any conclusions based on this observation. It was also found in the aggregation pheromones of spined soldier aphids, which also belong to Sternorrhyncha, the importance of chemoception in mate and host location in psyllids (Horton et al., 2007, 2008; Lapis and Borden, 1993; Mann et al., 2012; Patt and Sétamou, 2010; Wenninger et al., 2008; Yang et al., 1986). In aphids, which also belong to Sternorrhyncha, the importance of olfaction in host location has, however, been demonstrated in many species, and several SSR studies have been performed (Campbell et al., 1993; Hardie et al., 1995; Nottingham et al., 1998). One of the stereoisomers, (E,Z)-β-ocimene, myrcene, 2-hexenal and 6-methyl-5-hepten-2-one were recorded in the A neuron and/or in the B neuron (Table 2).

3.6. Temporal response pattern

The temporal response pattern was highly similar between different OSNs, compounds, and doses. The OSN response was always phasic-tonic. In S1, the B cell response to linalool started ca. 200 ms after the puff and peaked after ca. 400 ms. The weaker response in the A cell to β-caryophyllene peaked after ca. 600 ms. The phasic part of the response was followed by a sudden drop in spike activity, leading into a tonic response (starting ca. 1.5 s after the onset of stimulation) characterized by elevated (3–5 spikes/200 ms) but decreasing firing frequency until spontaneous activity was restored (Fig. 5A).

In S2, we analyzed how the temporal response profile was affected by the dose of Z3-hexenol. The phasic-tonic pattern remained for all doses, but the spike frequency and duration of the tonic part of the response decreased with decreasing dose (Fig. 5B).

In S3, we analyzed the temporal response profiles of the OSN when activated by 1-hexanol, Z3-hexenol and Z3-hexenyl acetate at the 10 µg dose. The response pattern was highly similar for the three compounds, all eliciting a phasic-tonic response (Fig. 5C). Responses peaked ca. 400 ms after the onset of stimulation, and then gradually decreased into a tonic response, similar to that of S1, starting ca. 1.5–2 s after the onset of stimulation. During the tonic response, the spike activity was ca. 5 spikes/200 ms, but slowly decreasing until resting activity was restored. The initial spike activity was slightly higher for Z3-hexenol than for 1-hexanol and Z3-hexenyl acetate. The temporal response profile of the B neuron in S2 was highly similar to that of S3 when stimulated with the same three compounds (data not shown).

4. Discussion

In this study, we characterized odor responses and found strongly activating ligands for OSNs in three of the four sensillar cavities of the blue gum psyllid C. eucalypti. To our knowledge, this is the first SSR study performed on eucalypt psyllids and the second considering the entire Psylloidea superfamily. Previously, SSR was performed on the carrot psyllid Triozia apicalis (Kristoffersen et al., 2008) and coupled gas chromatographic–electroantennographic detection (GC–EAD) on Cacopsylla bidens (Soroker et al., 2004). In addition, a few behavioral studies have shown the role of chemoreception in mate and host location in psyllids (Horton et al., 2007, 2008; Lapis and Borden, 1993; Mann et al., 2012; Patt and Sétamou, 2010; Wenninger et al., 2008; Yang et al., 1986). In aphids, which also belong to Sternorrhyncha, the importance of olfaction in host location has, however, been demonstrated in many species, and several SSR studies have been performed (Campbell et al., 1993; Hardie et al., 1995; Nottingham et al., 1991; Pickett et al., 1992; Pettersson et al., 1994; Powell and Hardie, 2001).

We showed, based on discernible spike amplitudes, that the olfactory sensilla of C. eucalypti contain three OSNs. However, only one or two OSNs (A and/or B cells) in each sensillum responded to the volatile stimuli they were exposed to. In addition, the neurons housed in sensillum S2 and S3 showed similar response profiles, indicating that the same odorant receptor(s) might be expressed in the B neurons in both sensilla. This distribution pattern of OSN types is similar to the distribution of OSNs in the T. apicalis, where some of the classified neuron types were found in two or more of the four sensillar cavities (Kristoffersen et al., 2008). Overall, the response spectra of male and female OSNs were very similar, but we observed some variation between sexes (Table 2). However, the gender variation was only minor and mainly evident among the compounds eliciting very weak responses. Therefore, it remains uncertain whether the variation has any ecological relevance and we do not draw any conclusions based on this observation.

The B neuron in sensillum S1 of C. eucalypti showed a strong response only to the oxygenated monoterpene linalool, suggesting that this OSN is highly selective for this general plant volatile. Linalool is known to play many roles in insects. For instance, it acts as a toxicant to a variety of insect species at high concentration (Abdelgaleil et al., 2009; Chang et al., 2009; Phillips et al., 2010). It was also found in the aggregation pheromones of spined soldier bugs, Podisus maculiventris and P. nigrispinus (Sant’anna and Dickens, 1998). One of the stereoisomers, (R)-(−)-linalool (licareol) was
reported as a short-range kairomone and synergist in both Tetropium fuscum and Anoplophora glabripennis (long horned beetles: Coleoptera) (Silk et al., 2010) whereas the other stereoisomer (S)-(-)-linalool (coriandrol) was reported as an attractive pheromone component in Colletes curricularis (a solitary bee: Hymenoptera) (Borg-Karlson et al., 2003). Conversely, linalool interferes with host finding by repelling economically important aphids such as Aphis fabae (black bean aphid), Caviaelli aegopodi (carrot aphid), Myzus persicae (green peach aphid) and Acrthosiphon pisum (pea aphid) (Bruce et al., 2005b; Chapman et al., 1981), which suggests the presence of linalool-detecting OSNs in these species too. The Asian citrus psyllid and the carrot psyllid responded behaviorally and physiologically, respectively, to linalool as well as to other mono- and sesquiterpenes, such as (E)-β-ocimene, β-caryophyllene, terpinene and terpen-4-ol (Kristoffersen et al., 2008; Patt and Sétemou, 2010). Since OSNs of C. eucalypti also responded to linalool, (E)-β-ocimene and β-caryophyllene, it is possible that terpene-detecting OSNs are a general characteristic of psyllid olfaction. Furthermore, our SSR and GC–SSR from the OSNs inside S1 demonstrate that differences in compound release rates, in this case linalool and β-caryophyllene, from Pasteur pipette stimulus cartridges can have large effects on the strength of the OSN responses. This was previously demonstrated in various other insect species (Andersson et al., 2012b). Furthermore, the GC–SSR also revealed that the A neuron in S1 was inhibited while the B neuron simultaneously responded to linalool, suggesting that lateral interactions occur between OSNs in the periphery, as previously found in other species (Andersson et al., 2010; Su et al., 2012).

In this study, C. eucalypti showed strong responses to GLV alcohols and acetates (1-hexanol, Z3-hexenol and Z3-hexenyl acetate) (Table 2). GLVs are produced in large amounts by angiosperm trees (Zhang and Schlyter, 2004), and most studied insect species have one or several classes of OSNs that are tuned to these compounds (e.g. Andersson et al., 2009; Bengtsson et al., 2009; Binyameen et al., 2012), sometimes very specifically (Andersson et al., 2012a; Hansson et al., 1999; Larsson et al., 2001). GLV compounds such as E2-hexenal, 1-hexanol, Z3-hexenol and Z3-hexenyl acetate are electrophysiologically active in many aphid species (Hardie et al., 1995; Visser and Piron, 1995; Visser et al., 1996). GLVs can act as attractants to some insect species (Visser and Avé, 1978), and as repellents to others, indicating the presence of unsuitable hosts (Unsicker et al., 2009; Zhang and Schlyter, 2004). It remains to be determined whether GLVs may attract or repel C. eucalypti. The weak response of the OSNs in S4 and the absence of responses in all C neurons suggest that these OSNs may detect plant compounds not tested in this study or possible pheromone compounds, if they exist for C. eucalypti.

Insect host location often relies on a tiny proportion of the compounds present in the host plant headspace. Thus, the headspace contains a surplus of compounds that are not used in host location (Birkett et al., 2004; Nojima et al., 2003; Tasin et al., 2007). This was clearly demonstrated in the present study, in which C. eucalypti was non-responsive or responded only weakly to many of the compounds in the odor panel in spite of the high screening dose (10 µg). In addition, the neurons in S1–S3 were highly confined in their response specificity, responding strongly to only one (S1) or three (S2 and S3) compounds. High specificity of plant odor-responding OSNs (see Mustaparta, 2002 for a discussion about specific and broadly tuned OSNs) is commonly found in insects and is considered integral to their capacity to identify host from non-host plant species (Andersson et al., 2009, 2012a; Hansson et al., 1999; Stensmyr et al., 2001; Wilbe and Mustaparta, 1996).

When comparing the organization of sensilla in C. eucalypti to that of T. apicalis (Kristoffersen et al., 2008), there is no difference in the number of sensilla and in their position on the different antennal filaments. However, the OSN response profiles varied between the two species. Triozoa apicalis responded to terpinene-4-ol in sensilla S1 and S4, and to terpinenol in S1 (Kristoffersen et al., 2008). Ctenarytaina eucalypti did not respond strongly to either terpinene-4-ol or terpinenol. On the other hand, OSNs in sensilla S2 and S3 of both species responded to chemically related GLVs, i.e. T. apicalis responded to Z3-hexenol and C. eucalypti responded primarily to the corresponding alcohol, Z3-hexenol. Thus, both psyllids have GLV-detecting OSNs in sensilla S2 and S3. Unfortunately, only Z3-hexenol was tested for T. apicalis, so we do not know whether the GLVs that elicited responses in C. eucalypti also are active on T. apicalis.

The OSNs in S1–S3 showed highly similar phasic-tonic response dynamics to different compounds and different concentrations (Fig. 4). The importance of the temporal structure of OSN responses is poorly understood, but might be associated with the ability of an insect to follow pulsed stimuli. Pulsed stimuli are thought to be
more easily tracked by phasic neurons, which are better at sensing rapid fluctuations in odor concentration, such as those occurring in natural odor plumes (Almaas et al., 1991; Murlis et al., 2000). In contrast, tonic responses might provide short-term memory of recently encountered odor stimuli (de Bruyne et al., 2001; Den Otter and Van der Goes van Naters, 1992). Temporal variation in OSN responses has been studied also in other insects. However, in contrast to the present study in which all the analyzed OSNs responded similarly, differences in temporal response dynamics have been recorded for different compounds, OSN classes, stimulus concentrations (Andersson et al., 2012a), or between OSNs located on different parts of the antenna (Binyameen et al., 2012). The behavioral or ecological consequences of these differences are unknown, but should be the focus of forthcoming investigations.

The compounds that elicited responses in this study will be tested for behavioral activity. Possible attractants or repellents may be useful for monitoring and control of C. eucalypti populations. Insect host location can be mediated by a single compound (Guerin et al., 1983; Hern and Dorn, 2004), for instance when plants emit taxonomically specific volatiles (Bruce et al., 2005a).

Fig. 5. Temporal response characteristics of Ctenarytaina eucalypti olfactory sensory neurons (OSNs). Left panel: representative spike trains. Right panel: temporal response curves obtained by counting spikes in 200 ms bins and subtracting the spontaneous activity, i.e. average spike activity during the 2 s before the response. Black horizontal bars indicate the 0.5 s odor puffs. (A) The A and B neurons present in S1 responded phasic-tonic to β-caryophyllene and linalool, respectively, at the 10 µg dose on the filter paper (N = 5). (B) The phasic-tonic response to different doses of Z3-hexenol in B neurons in S2 (N = 3). (C) The phasic-tonic response of B neurons in S3 to Z3-hexenol, Z3-hexenyl acetate, and 1-hexanol at the 10 µg dose (N = 5).
However, in this study we found strong responses to common plant volatiles, and no response to 1,8-cineole (eucalyptol) that dominates the eucalypt headspace. Moreover, since many eucalypts, especially those within the same subgenus, share so many terpene compounds (discussed in Paine et al., 2011), host location by C. eucalypti may be influenced by specific volatile mixtures and/or by other host cues (e.g. visual stimuli) that characterize the preferred species and leaf type (juvenile or adult). The relative influences of these different cues and mechanisms are the focus of on-going behavioral studies.

In conclusion, the current study characterized the specificity of plant odor-detecting OSNs in the blue gum psyllid. Even though more studies need to be conducted to identify the functions of the active compounds identified here, the knowledge gained from this study contributes to our understanding of the chemical ecology of psyllids.

Acknowledgements

M.J.S thanks Bronwyn Meredith (Wildlife Trade Assessments, Department of Sustainability, Environment, Water, Population and Communities, Canberra) for assistance obtaining insect export permits and the Australian Research Council for Future Fellowship funding (FT100100199). K.F. was funded by an Australian Postgraduate Award from the Australian Government with top-up funding provided by La Trobe University. The authors would like to thank Jan-Robert Baars (School of Biology & Environment Science, University College Dublin, Ireland) for also collecting and shipping psyllids for this study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jiphys.2013.03.004.

References


