Journal of Insect Physiology 55 (2009) 556–567

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Specificity and redundancy in the olfactory system of the bark beetle *Ips typographus*: Single-cell responses to ecologically relevant odors

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ARTICLE INFO

Article history:
Received 9 December 2008
Received in revised form 29 January 2009
Accepted 30 January 2009

Keywords:
Single-sensillum recordings
Odor coding
Non-host volatiles
Olfactory receptor neuron
Host selection

ABSTRACT

We screened 150 olfactory sensilla in single-sensillum recordings to unravel the mechanisms underlying host selection in the spruce bark beetle, *Ips typographus* (Coleoptera: Curculionidae: Scolytinae). Odor stimuli comprised of pheromone (various bark beetle spp.), host, and non-host compounds elicited strong and selective responses from 106 olfactory receptor neurons (ORNs), 45 of which were tuned to pheromone compounds, 37 to host compounds, and 24 to non-host volatiles (NHV). In addition, 26 ORNs responded only weakly to any odor stimulus. Strongly responding ORNs were classified into 17 classes. Seven classes responded primarily to the *Ips* pheromone compounds: *cis*-verbenol, ipsenol, ipsdienol (two classes), 2-methyl-3-buten-2-ol, amitinol, or verbenone, respectively. Six classes responded to the host compounds: α-pinene, myrcene, *p*-cymene, myrcene and *p*-cymene, 1,8-cineole, or Δ3-carene, respectively. Four classes responded to NHV: 3-octanol, 1-octen-3-ol, *trans*-conophthorin, or indiscriminately to the repellent green leaf volatiles (GLVs) 1-hexanol, *Z*3-hexen-1-ol and *E*2-hexen-1-ol, respectively. Indiscriminate responses from GLV neurons might explain a behavioral redundancy among these GLVs. This is the first description of individual bark beetle ORNs dedicated to NHV perception. These comprise almost 25% of the strongly responding neurons, demonstrating that a large proportion of the olfactory system is devoted to signals from plants that the insect avoids.

1. Introduction

The European spruce bark beetle, *Ips typographus* (Coleoptera: Curculionidae: Scolytinae) attacks and kills Norway spruce, *Picea abies*, in Europe. This has stimulated a large amount of research concerning its olfactory-mediated host selection behavior. Attraction to host trees is governed by secondary attraction of both sexes to the male-produced aggregation pheromone that consists of (4*S*)-(−)-*cis*-verbenol and 2-methyl-3-buten-2-ol (Schlyter et al., 1987a). Whether the pioneering males locate host trees at random or if primary attraction (in the absence of pheromone) to host odors (kairomone) also occurs has not been clearly demonstrated, but spruce volatiles, particularly in large amounts, seem to improve the attraction to the pheromone bait (Jakus and Blazenec, 2003; Hulcr et al., 2006; Erbilgin et al., 2007). The *Ips*-associated compounds, verbenone, *E*-myrcenol, ipsenol and high doses of ipsdienol, as well as the pheromone components of the sympatric *Pityogenes chalcographus*, (1*S*,5*R*)-chalcogran and methyl (*E*,*Z*)-2,4-decadienoate, inhibit the attraction of *I. typographus* (Schlyter et al., 1989, 1992; Byers, 1993) and likely mediate avoidance of intra- and interspecific competition.

Behavioral responses are also modulated by non-host volatiles (NHV) that originate from angiosperm trees, and anti-attractant effects of various NHV have been demonstrated in more than 20 bark beetle species (reviewed by Zhang and Schlyter, 2004). For the spruce bark beetle, the green leaf volatiles (GLVs) 1-hexanol, *E*2-hexen-1-ol and *Z*3-hexen-1-ol have inhibitory properties (Zhang et al., 1999). In addition, 3-octanol, 1-octen-3-ol, and the spiroacetal (5*S*,7*S*)-*trans*-conophthorin (Zhang et al., 2002), isolated from bark of angiosperm trees (*Betula* and *Populus* spp.), inhibit the attraction to the pheromone (Zhang et al., 2000). The chemical ecology of *I. typographus* is complex, with behavioral responses to binary and ternary blends of inhibitory chemicals involving both redundancy and synergism (Zhang and Schlyter, 2003). Redundancy occurs among and between the GLV alcohols and the C_8-alcohols, whereas *trans*-conophthorin and verbenone synergize the effect of the alcohols.

Previous studies identified olfactory receptor neurons (ORNs) specialized to several bark beetle pheromone compounds and a few host monoterpenes (Mustaparta et al., 1984; Tømmerås et al.,

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doi:10.1016/j.jinsphys.2009.01.018

Table 1
Classifications, origins, and behavioral effects of the chemicals used in the study.

Chemical	Abbreviation	Chemical source	Purity %	Chemical class	Biological class	Behavioral effect	Ecological source	Refs
(−)-*cis*-Verbenol	cV	Borregaard	95	MT–OH	Ph	++	*I. typographus* aggr. Ph	Schlyter and Birgersson (1999)
(−)-*trans*-Verbenol	tV	SciTech Ltd., Prague	91	MT–OH	Ph	?	*Tomicus* spp. Ph	Schlyter and Birgersson (1999)
(−)-Verbenone	Vn	Fluka	>99	MT=O	Ph	–	*I. typographus* anti-aggr. Ph	Schlyter and Birgersson (1999), Schlyter et al. (1989)
(±)-Ipsenol	Ie	Bedoukian	97 (95)[b]	MT–OH	Ph	–	*I. typographus* anti-aggr. Ph	Schlyter and Birgersson (1999), Schlyter et al. (1989)
(±)-Ipsdienol	Id	Bedoukian	94 (95)	MT–OH	Ph	–+	*Ips* spp. Ph	Schlyter and Birgersson (1999), Schlyter et al. (1989)
Amitinol	Am	W.F.[a]	96	MT–OH	Ph	?	*Ips* spp. Ph	Zhang et al. (2007)
E-Myrcenol	EM	SciTech Ltd., Prague	94 (95)	MT–OH	Ph	–	*Ips* spp. Ph	Schlyter and Birgersson (1999), Schlyter et al. (1992)
2-Methyl-3-buten-2-ol	MB	Acros	>99 (97)	HT–OH	Ph	++	*I. typographus* aggr. Ph	Schlyter and Birgersson (1999)
(±)-Chalcogran	Cg	Celamerck	90 (93)	C_9–spiroacetal	Ph	–	*P. chalcographus* Ph	Schlyter and Birgersson (1999), Byers (1993)
(±)-*exo*-Brevicomin	xB	W.F.	99	C_9–diacetal	Ph	+	*Dendroctonus* spp. Ph	Schlyter and Birgersson (1999), Tømmerås and Mustaparta (1984)
Methyl (*E*,*Z*)-2,4-decadienoate	MD	Bedoukian	92	C_{10}–O–Me	Ph	–	*P. chalcographus* Ph	Schlyter and Birgersson (1999), Byers (1993)
(+)-α-Pinene	(+)αP	Janssens chimica	98	MT	Host	?	Spruce	Persson et al. (1996)
(−)-α-Pinene	(−)αP	Fluka	>99	MT	Host	+	Spruce. Attractive with Ph	Persson et al. (1996), Erbilgin et al. (2007)
(−)-β-Pinene	βP	Fluka	92 (>99)	MT	Host	?	Spruce	Persson et al. (1996)
Myrcene	My	Fluka	95	MT	Host	?	Spruce	Persson et al. (1996)
p-Cymene[a]	pC	Acros	>99	MT	Host		Spruce	Persson et al. (1996), Schlyter (unpublished data)
(+)-Limonene	(+)L	Fluka	>99	MT	Host	?	Spruce	Persson et al. (1996)
(−)-Limonene	(−)L	Fluka	98 (>99)	MT	Host	?	Spruce	Persson et al. (1996)
(±)-Δ3-Carene	Δ3	Aldrich	93 (95)	MT	Host	?	Pine > spruce	Persson et al. (1996), Wibe et al. (1998)
(±)-1,8-Cineole	Ci	Aldrich	>99	MT–acetal	Host	–	Spruce	Schlyter (unpublished data), Wajs et al. (2006)
Terpinolene	Te	Fluka	97	MT	Host	?	Pine > spruce	Persson et al. (1996)
(−)-Bornyl acetate	BoAc	Acros	>99 (97)	MT–OAc	Host	?	Spruce	Wajs et al. (2006)
1-Hexanol	C6	Fluka	>99	C_6–OH	NHV	–	GLV	Zhang and Schlyter (2004)
E2-Hexen-1-ol	E2C6	Acros	93 (96)	C_6–OH	NHV	–	GLV	Zhang and Schlyter (2004)
Z3-Hexen-1-ol	Z3C6	Acros	98	C_6–OH	NHV	–	GLV	Zhang and Schlyter (2004)
Hexanal	C6Ald	Aldrich	98	C_6=O	NHV	0	GLV	Zhang and Schlyter (2004)
E2-Hexenal	E2C6Ald	Aldrich	95 (98)	C_6=O	NHV	0	GLV	Zhang and Schlyter (2004)
Z3-Hexenyl acetate	Z3C6Ac	Sigma	99	C_6–OAc	NHV	0	GLV	Zhang and Schlyter (2004)
(±)-3-Octanol	C8an	Acros	>99	C_8–OH	NHV	–	Non-host bark	Zhang and Schlyter (2004)
(+)-3-Octanol	(+)C8an	Aldrich	98 (97)	C_8–OH	NHV	?	Enantiomeric composition in bark unknown	
(±)-1-Octen-3-ol	C8en	Acros	98	C_8–OH	NHV	–	Non-host bark	Zhang and Schlyter (2004)
(−)-1-Octen-3-ol	(−)C8en	Acros	98 (99)	C_8–OH	NHV	?	Enantiomeric composition in bark unknown	
5*S*,7*S*-*trans*-Conophthorin	SStC	W.F.	92 (97)	C_9–spiroacetal	NHV	–	Non-host bark, predominant enantiomer	Zhang and Schlyter (2004), Zhang et al. (2002)
5*R*,7*R*-*trans*-Conophthorin	RRtC	W.F.	74 (91)	C_9–spiroacetal	NHV	?	Non-host bark	Zhang and Schlyter (2004), Zhang et al. (2002)
(±)-Linalool	Lol	Fluka	92 (97)	MT–OH	NHV	0	Many plants	Zhang and Schlyter (2004)
(−)-Linalool	(−)Lol	Fluka	94 (>98)	MT–OH	NHV	?	Many plants	Zhang and Schlyter (2004)

Note: Compounds classified as ‘host’ can typically also be found in plants other than spruce (mainly conifers). MT: monoterpene (10C), HT: hemiterpene (5C), –OH: alcohol, =O: aldehyde and ketone, –Me: methyl, –OAc: acetate ester, Ph: pheromone (i.e. bark beetle-produced), NHV: non-host volatile, GLV: green leaf volatile.

[a] Wittko Francke, University of Hamburg, Hamburg, Germany.

[b] All compound purities were analysed with GC–MS. Parenthesis indicates purity specified on vial if different from our analysis.