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Review Article

Mechanisms of Odor Coding in Coniferous Bark Beetles: From Neuron to Behavior and Application

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Coniferous bark beetles (Coleoptera: Curculionidae: Scolytinae) locate their hosts by means of olfactory signals, such as pheromone, host, and nonhost compounds. Behavioral responses to these volatiles are well documented. However, apart from the olfactory receptor neurons (ORNs) detecting pheromones, information on the peripheral olfactory physiology has for a long time been limited. Recently, however, comprehensive studies on the ORNs of the spruce bark beetle, *Ips typographus*, were conducted. Several new classes of ORNs were described and odor encoding mechanisms were investigated. In particular, links between behavioral responses and ORN responses were established, allowing for a more profound understanding of bark beetle olfaction. This paper reviews the physiology of bark beetle ORNs. Special focus is on *I. typographus*, for which the available physiological data can be put into a behavioral context. In addition, some recent field studies and possible applications, related to the physiological studies, are summarized and discussed.

1. Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) constitute some of the most destructive pests of coniferous trees throughout the world, destroying forests of great economic value. Currently, the large-scale outbreak of the mountain pine beetle, *Dendroctonus ponderosae*, in North America has resulted in the loss of hundreds of millions m^3 timber and turned the forests into major sources of carbon release [1]. In Europe and parts of Asia [2, 3], the European spruce bark beetle, *Ips typographus* (Figure 1), is considered the most destructive bark beetle of coniferous forests [4, 5].

Bark beetles, like most insects, locate their hosts mainly by means of olfactory signals. It is clear that they utilize both attractants and antiattractants that emanate from host and nonhost plants, as well as from conspecific and heterospecific bark beetle individuals [3, 6–10]. The odor molecules are transported downwind from their source of release as an odor plume with a complex structure [11–13]. Molecules are picked up by olfactory receptors (ORs) or ionotropic receptors (IRs) [14], located mainly in the antennae and maxillary palps. Specifically, the ORs are present in the cell membrane of olfactory receptor neuron (ORN) dendrites that, in turn, are housed within olfactory sensilla [15]. The ORs are encoded by a large and diverse family of olfactory receptor genes [16]. Each ORN is generally thought to express only one member from this family in addition to the widely expressed coreceptor, Orco [17]. IRs act in combinations of up to three subunits that are comprised of odor-specific receptors and one or two broadly expressed coreceptors [14]. These receptors are expressed in neurons that do not express ORs. When an odor molecule binds to a receptor, the ORN sends a neuronal signal to the primary olfactory center of the brain, the antennal lobe. Typically, the signal that is generated by an ORN is an increase in the firing frequency of action potentials (excitation), but some odorants may instead cause a decrease in firing activity (inhibition). ORNs can be divided into classes based on their odor response profiles. Often, ORNs are fairly specific and activated by only one or a few compounds, but some appear to have a broader tuning. In addition, each compound often activates more than one type of ORN, and thus, the odor input is thought to be constructed as a combinatorial code [18].

Figure 1: The European spruce bark beetle, *Ips typographus*. Photo: Göran Birgersson.

In contrast to the well-studied chemical ecology of bark beetles, until recently, little was known about the physiological responses of individual bark beetle ORNs. Mainly in the 1980s, Single-sensillum recordings (SSR) were carried out, primarily identifying classes of ORNs that responded to various pheromone compounds. Some decades later, comprehensive studies on *I. typographus* have characterized additional ORNs that respond also to host and nonhost plant compounds [19] and have provided novel insights into potential odor coding mechanisms in insects in general [8]. This review summarizes the results from early and recent studies on the physiology of ORNs in conifer-feeding bark beetles. Particular focus is on *I. typographus,* for which a sufficient amount of information has emerged in order to bridge the physiological data with previously recorded behavioral responses to several semiochemicals. In addition, some recent behavioral studies with connections to olfactory physiology are summarized and possible applications discussed. First, however, a brief overview of the semiochemicals that are used by *I. typographus* in host selection is presented.

2. Host Selection by *I. typographus*

The male is the initial host seeking, or "pioneering," sex of *I. typographus*. Once a male has located a suitable host material to colonize, it releases an aggregation pheromone, a mixture of (4*S*)-*cis*-verbenol and 2-methyl-3-buten-2-ol [20], which attracts individuals of both sexes. Although the olfactory-mediated host location behavior of *I. typographus* has been extensively studied, it is not known how the pioneering males locate a suitable host tree, as no primary attraction (in the absence of pheromone) to spruce volatiles has been demonstrated. However, spruce volatiles may modulate the pheromone response [9] or possibly attract beetles to a suitable habitat [21]. It is also possible that pioneering beetles land randomly on trees and assess host quality upon contact [22]. However, apart from the few pioneering males, the aggregation pheromone attracts the majority of individuals to the host.

The attraction to the pheromone is modulated by other semiochemicals that appear in later attack phases. Verbenone and ipsenol are two such compounds that are believed to be used as cues to avoid heavily attacked trees [23]. In addition, volatiles that are particularly abundant in nonhost angiosperm plants (so called nonhost volatiles, NHV), such as green leaf volatiles (GLVs) [24] and compounds from the bark, such as C8-alcohols and *trans*-conophthorin [25, 26], have inhibitory effects on pheromone attraction. Combining these compounds with verbenone produces a strong synergistic effect and a potent antiattractant blend [27]. Possibly, the individual constituents in the synergistic blend represent different levels in the host selection sequence [6]. The GLVs that are common to broad-leaved plants may represent a signal of a nonhost dominated habitat. More specific plant volatiles, such as *trans*-conophthorin, may indicate nonhosts at the tree species level [7], whereas the antiattractive pheromone components may signal unsuitability of individual spruce trees.

3. Olfactory Receptor Neurons of *I. typographus* and Other Bark Beetles

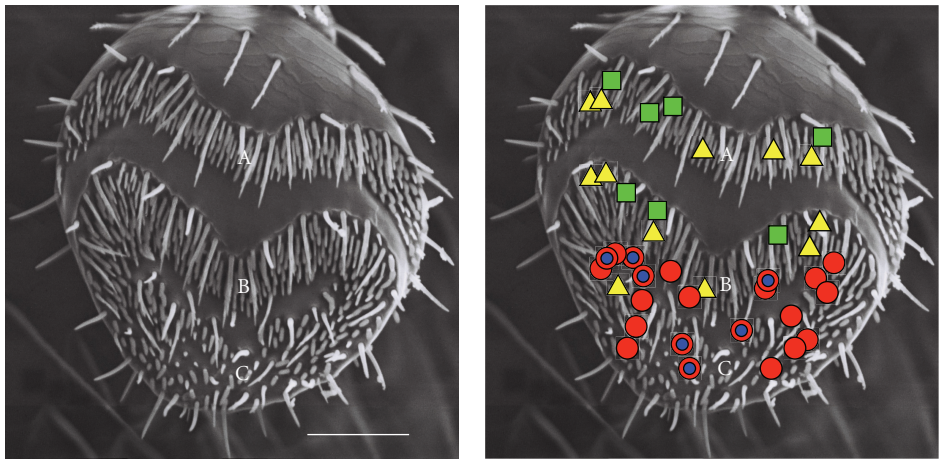
Many compounds that are either attractants or antiattractants for conifer bark beetles have been identified [3, 6, 7]. Single-sensillum recordings from the ORNs of several bark beetle species have shown that many of the behaviorally active compounds elicit responses in different classes of neurons (Table 1). It is obvious that, except for *I. typographus* and the ambrosia beetle *Trypodendron lineatum*, more is known about ORN responses to pheromone components than about responses to plant odors (Table 1). In addition, several of the tested compounds (i.e., ipsdienol, ipsenol, verbenone, *cis*/*trans*-verbenol, *exo*-brecicomin, and α-pinene) elicit strong responses in the majority of species studied. For more details on ORN specificity, sensitivity, and abundance in each species, the reader is referred to the cited literature.

Olfactory sensilla of *I. typographus* are present in three areas (or bands) on the antenna (Figure 2(a)) [41]. Andersson et al. [19] screened 150 olfactory sensilla for responses to an odor panel comprised of similar numbers of synthetic pheromone, host, and nonhost compounds. Strong excitatory responses were obtained from 106 ORNs; 45 responded specifically to various bark beetle pheromone compounds, 37 to host compounds, and 24 to antiattractive nonhost volatiles (NHVs). Based on response spectra, the 106 ORNs were grouped into 17 different classes (Figure 3). Additionally, 26 neurons (divided into 12 ORN classes) responded only weakly to any test odorant, indicating that the most potent compounds for these ORNs were lacking. In addition to the ORN classes described by Andersson et al. [19], three other classes, responding specifically to (+)-*trans*-verbenol, phenylethanol, or campher plus pino-camphone, respectively, had been identified previously (Table 1) [28]. Furthermore, the majority of the ORN classes responding to pheromone compounds was found in both studies. Many ORN classes have been subjected to dose-response trials that indicated that the ORNs, in general, are highly sensitive and specific for only one or a few structurally related pheromone or plant compounds (Figures 4(a)–4(c)) [19, 28]. Response thresholds for the best ligand(s) were normally found around

Table 1: Compounds from different ecological sources that elicit strong responses in olfactory receptor neurons in eight species of Scolytinae.

Species	Beetle-produced compounds	Host compounds	Nonhost compounds	References
Ips typographus	(+)-ipsdienol (−)-ipsdienol (−)-ipsenol (−)-*cis*-verbenol (+)-*trans*-verbenol (−)-verbenone 2-methyl-3-buten-2-ol Amitinol Phenylethanol *exo*-brevicomin (A)* (±)-chalcogran (A)	Myrcene Campher (B) Pino-camphone (B) *p*-cymene 3-carene 1,8-cineole (+)-*α*-pinene (C) (−)-*α*-pinene (C)	Pine bark extract Birch bark extract 1-hexanol (D) *E*2-hexenol (D) *Z*3-hexenol (D) 1-octen-3-ol 3-octanol (*S*,*S*)-*trans*-conophthorin (A)	[19, 28–31]
Ips pini	(+)-ipsdienol (−)-ipsdienol (±)-ipsenol *cis*-verbenol *trans*-verbenol Verbenone	Linalool Camphor Myrcene		[29, 32–34]
Ips paraconfusus	(+)-ipsdienol (−)-ipsdienol (±)-ipsenol			[33]
Dendroctonus pseudotsugae	Frontalin 3-methyl-2-cyclohexenone 3-methyl-2-cyclohexenol 1-methyl-2-cyclohexenol *trans*-verbenol *cis*-verbenol Verbenone Ipsenol	*α*-pinene Limonene		[35, 36]
Dendroctonus frontalis	(−)-frontalin *exo*-brevicomin *endo*-brevicomin Verbenone *trans*-verbenol	*α*-pinene 3-carene		[37, 38]
Dendroctonus micans	(+)-ipsdienol *exo*-brevicomin			[31]
Trypodendron lineatum	(+)-lineatin Phenylethanol	Ethanol Methanol Butanol *α*-pinene *β*-pinene Spruce bark extract	Pine bark extract Birch bark extract	[39]
Tomicus destruens		Compounds in pine extract	Benzyl alcohol	[40]

*Compounds that elicit responses of similar strength in the same ORN class are indicated by the same capital letter. Odorants eliciting secondary responses are omitted for clarity.



(a) (b)

Figure 2: (a) Olfactory sensilla are present in three areas (A, B, and C) on the antennal club of *Ips typographus*. (b) Spatial distribution patterns of four classes of olfactory receptor neurons (ORNs). ORNs responding to green leaf volatile alcohols (nonhost) = green squares, myrcene (host) = yellow triangles, *cis*-verbenol (pheromone) = red circles, 1,8-cineole (host) = blue small circles (from [19], with permission from the publisher). Scale bar = 50 μm.

the 1 ng dose on the filter paper using paraffin oil as solvent [19]. A high specificity, not only among pheromone ORNs, but also among those for plant compounds, seems to be a general rule also in other bark beetle species (see especially [32, 35]).

The ORNs of *I. typographus* are not randomly distributed on the antenna. Instead, ORNs from a particular class are generally found either in both the proximal and medial bands of sensilla, or exclusively in the distal area (Figure 2(b)) [19]. This distribution pattern seems to correspond to the distribution of the two morphological types of single-walled sensilla previously identified [41].

It is common in insects that the pheromone ORNs are numerous on the antenna and that the most common ORN type is tuned to the major (most abundant) component [42, 43]. In *I. typographus*, the most recurrent ORN class was tuned to (−)-*cis*-verbenol [19]. In contrast, there were only few cells specific for 2-methyl-3-buten-2-ol (MB) (Figure 3) [19, 28], an essential pheromone component which is produced, and behaviorally active, in much larger quantities [20, 44]. This suggests that the pattern might be reversed in the bark beetle. However, the MB cells were found in a restricted area on the antenna [19], that is, on the borderline between the medial band and distal area of sensilla, which could have resulted in this cell type being underrepresented among the sampled sensilla. Alternatively, as MB is highly volatile, the low number of cells could be the result of the compound being lost from the stimulus cartridge upon stimulation. Indeed, photoionization detector measurements showed that the airborne amount of MB released from the stimulus pipette drops dramatically upon stimulation (Figure 5) [45]. However, the insect ORN still responded vigorously despite the low concentration, rendering this explanation unlikely. In contrast to Andersson et al. [19], Tømmerås [28] found that the ORNs tuned to ipsdienol were the most common ones

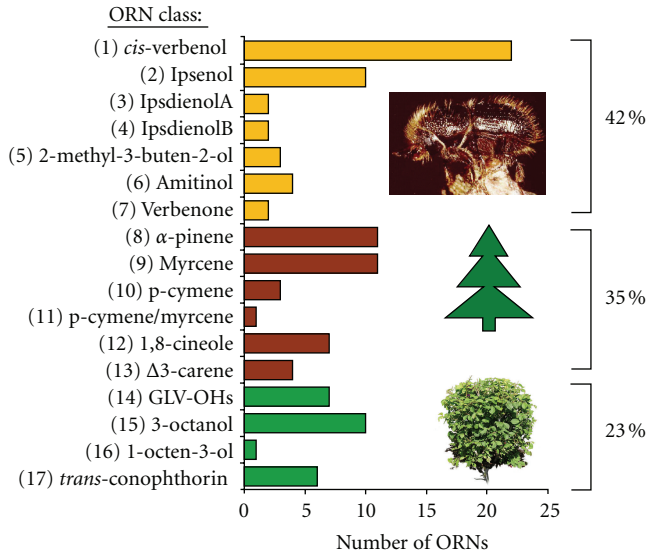


Figure 3: Number of olfactory receptor neurons (ORNs) of 17 strongly responding classes of *Ips typographus* (data from [19]). ORN classes are labeled according to which compound(s) elicited the strongest response. As pure enantiomers were not tested, it is likely that the ipdienol ORN classes A and B correspond to the ORNs responding to (+)- and (−)-ipdienol, respectively [29]. Orange = bark beetle pheromone compounds, brown = conifer compounds, green = nonhost volatiles. GLV-OHs = green leaf volatile alcohols.

in *I. typographus*. This discrepancy may also be explained by the nonrandom localization of ORNs on the antenna (i.e., neurons for *cis*-verbenol are abundant only in the distal part of the antennae, Figure 2(b)).

Although no primary attraction has been demonstrated, the high frequency of ORNs tuned to conifer-related

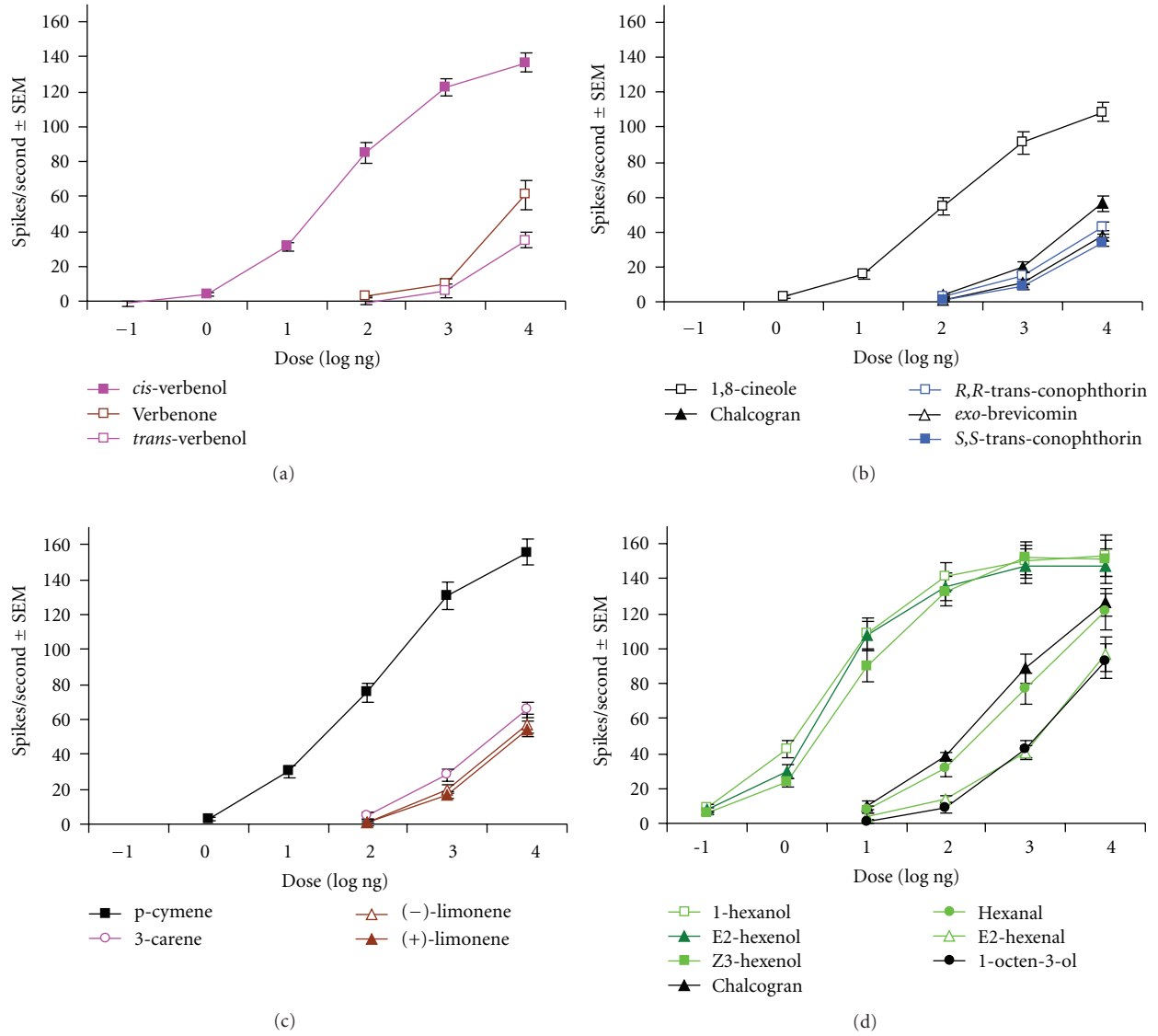


FIGURE 4: Dose-response curves from four receptor neuron classes of *Ips typographus*, demonstrating specific primary responses to (a) the pheromone component *cis*-verbenol, the spruce compounds (b) 1,8-cineole and (c) p-cymene. (d) Indiscriminate response to the three green leaf volatiles 1-hexanol, *E*2-hexenol, and *Z*3-hexenol (modified from [19], with permission from the publisher).

monoterpenes (Table 1, Figure 3) suggests that host kairomone is relevant for host location by *I. typographus*. As mentioned previously, these compounds may serve as habitat-scale attractants [21], or as modulators of pheromone attraction [8, 9]. Perhaps the most striking finding from the bark beetle SSR studies is that almost 25% of the strongly responding ORNs were specifically tuned to anti-attractive NHV (Table 1, Figure 3) [19]. This indicates that insects may devote a lot of olfactory capacity to the detection of compounds from sources that they avoid. Similar results have not been found in any other insect studied so far, however, it is likely that many other bark beetles that show strong GC-EAD responses to NHV also have a large proportion of ORNs tuned to such compounds [7, 46–48].

4. Discrimination of Enantiomers

Most bark beetle pheromone compounds are chiral. Attraction is typically evoked by only one of the enantiomers, while the other sometimes inhibits attraction (e.g., [20, 33]). The enantiospecific behavioral response is reflected in the specificity of the ORNs detecting the compounds (Table 1). For instance, ORNs that are specific for either the (+)- or the (−)-enantiomer of ipsdienol, ipsenol, verbenone, or *cis*- and *trans*-verbenol have been identified in several *Ips* species [28, 33]. Other examples are the ORNs in the Southern pine beetle, *D. frontalis* [37], and in the Douglas-fir beetle, *D. pseudotsugae* [36], that discriminate between the (+)- and (−)-enantiomer of frontalin (Table 1).

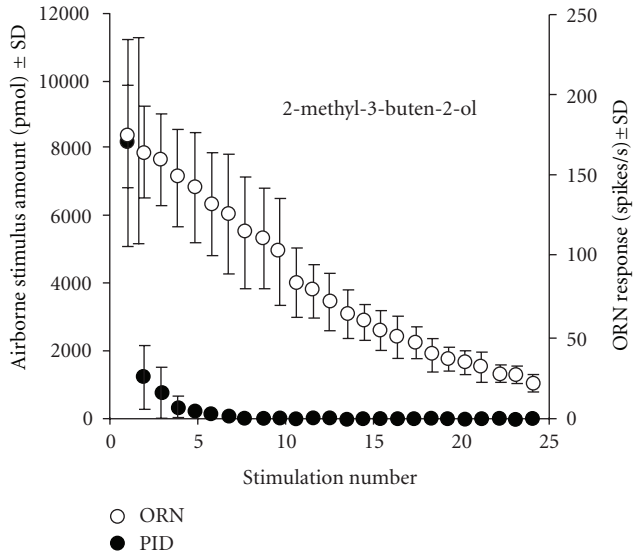


Figure 5: Response of *Ips typographus* olfactory receptor neurons (ORNs) and a photoionization detector (PID) to successive stimulations with 2-methyl-3-buten-2-ol ($N = 4$) (modified from [61]).

In general, the sensitivity of the pheromone ORNs seems to be 10–100-fold higher for the enantiomer that they are tuned to, as compared to the other [28, 33]. In addition, there seems to be a correspondence between the attraction to a specific enantiomer, and the frequency of ORNs on the antenna that responds to it. For instance, *I. pini* is attracted by (−)-ipsdienol and has more of its ORNs tuned to (−)-ipsdienol than to (+)-ipsdienol. Similarly, *I. paraconfusus*, which is attracted by (+)-ipsdienol, has most of its ipsdienol ORNs tuned to the (+)-enantiomer [33].

Enantiospecific responses to plant compounds have been recorded in *I. typographus* [19]. The neuron class that responded most strongly to the nonhost volatile *trans*-conophthorin (Table 1) was >100-fold more sensitive to the (5*S*,7*S*)-enantiomer than to the (5*R*,7*R*)-enantiomer. In fact, other structurally related compounds (racemic *exo*-brevicomin and chalcogran) elicited stronger responses in this ORN than did the (5*R*,7*R*)-enantiomer of *trans*-conophthorin [19]. In another class of ORN, the naturally occurring (−)-1-octen-3-ol elicited a slightly stronger response than the racemic mixture, indicating that the (−)-enantiomer is the key ligand. In contrast, the neuron that is tuned to α-pinene responded similarly to both enantiomers (Table 1) [19].

5. Olfactory Receptor Neuron Responses and Behavior

The results that have been obtained from single sensillum recordings [19, 28] indicate that behavioral responses of *I. typographus* to several compounds can likely be explained by the responses of the ORNs.

Several volatiles from nonhost plants were previously shown to inhibit pheromone attraction of *I. typographus* [24–26]. The three GLVs: 1-hexanol, *E*2-hexenol, and *Z*3-hexenol all reduced pheromone attraction to a similar extent. However, combining the three did not produce a stronger inhibition of attraction, a phenomenon defined as redundancy [27]. Interestingly, the only ORN that was sensitive to any of these volatiles had a more or less identical sensitivity to all three of them (Table 1, Figure 4(d)) [19]. Thus, it appears as if the bark beetle cannot differentiate between the compounds at the physiological level, which agrees well with their behavioral redundancy. In contrast, the compounds verbenone and *trans*-conophthorin that synergize the inhibition are detected by different ORNs [19, 28]. Interestingly, the pheromone component, chalcogran, of the sympatric *Pityogenes chalcographus*, was primarily detected, by *I. typographus*, by the same neuron as *trans*-conophthorin (Table 1). Chalcogran also inhibits pheromone attraction of *I. typographus* [49].

Most insects house their ORNs for pheromone compounds in sensilla that are distinct from the ones that detect plant compounds (e.g., [50, 51]). However, in some sensilla in *I. typographus,* the ORN for the aggregation pheromone component *cis*-verbenol (cV) is colocalized with an ORN that responds to the host plant compound 1,8-cineole (Ci) [8, 19] (Figure 2(b)). This lack of segregation between ORNs detecting pheromones and plant volatiles may suggest that host finding in bark beetles is an integrated process that involves both pheromones and plant volatiles. When the ORN for Ci responded, the colocalized cV cell was inhibited, indicative of interactions between ORNs in the periphery. In addition, Ci was found to be particularly abundant in heavily attacked spruce trees and the compound strongly reduced pheromone attraction (88% reduction in trap catch) in the field [8]. Possibly, Ci is a signal of an unsuitable (crowded) host or a well-defended tree.

6. Peripheral Modulation of ORN Responses

Colocalization of insect ORNs in the same sensillum is thought to improve coincidence detection, which increases the insect's spatiotemporal resolution of odor signals [52] and improves ratio detection of ecologically relevant odor mixtures [53]. In addition, the presence of two or more neurons in the same sensillum may provide opportunities for signal modulation in the periphery. Indeed, in the Douglas-fir beetle, *Dendroctonus pseudotsugae*, two ORNs, each specific for one of the two pheromone components 3-methyl-2-cyclohexenone or 3-methyl-2-cyclohexenol, are colocalized. When either one of the ORNs responded to its specific ligand, the spontaneous activity of the other ORN was reduced. This observation indicated reciprocal interactions, either directly between the two neurons, or between the two ligands and their respective receptors [35]. In addition, when another ORN type that responded to limonene (10 ng dose) was challenged with a binary mixture of limonene and 3-methyl-2-cyclohexenol (10 : 1000 ng), the response to limonene was completely shut down [35].

In *I. typographus*, not all cV neurons (large amplitude A-cell) are colocalized with the neuron for Ci (small amplitude B-cell) (Figure 6(a)). These cV neurons are instead found

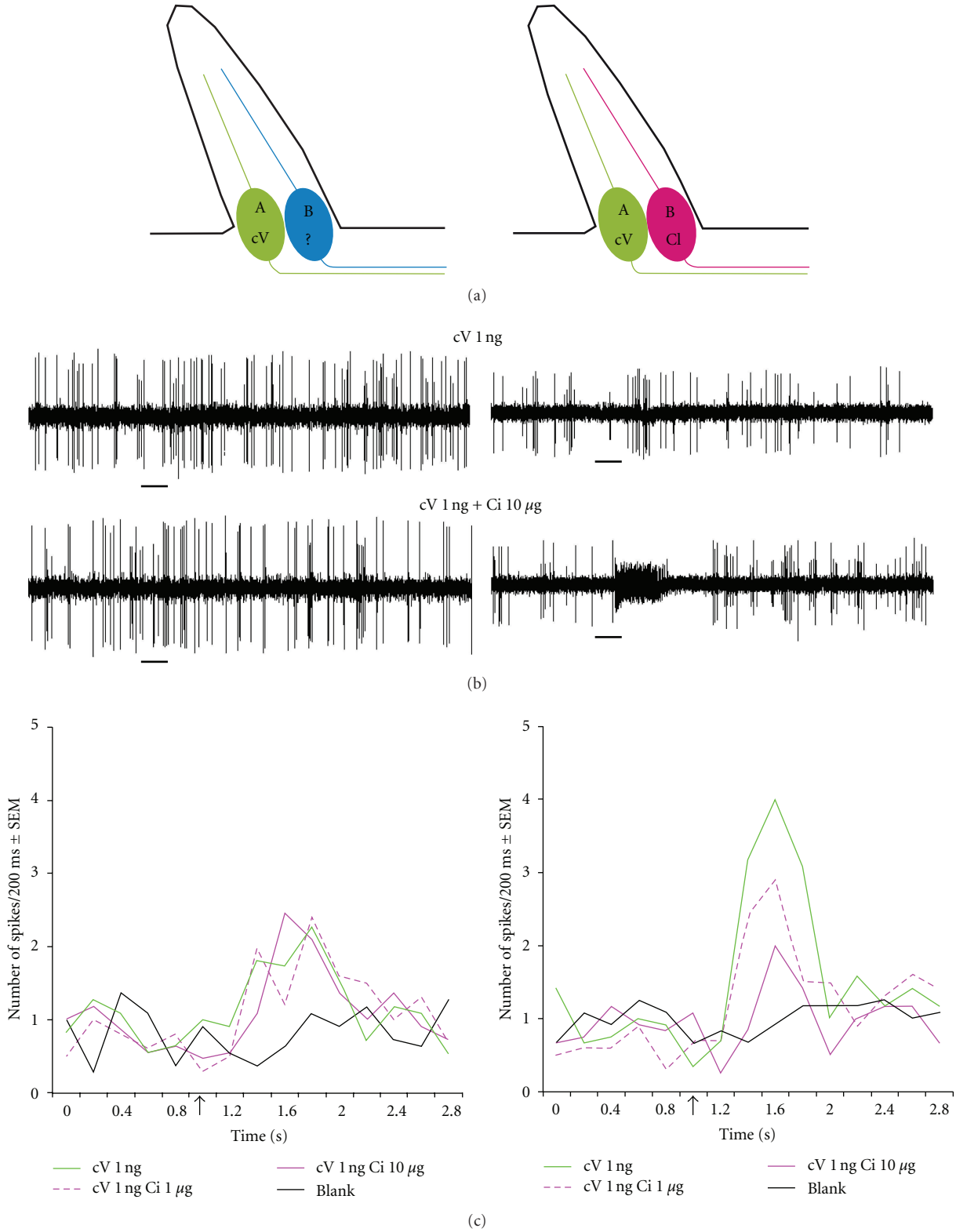


Figure 6: (a) Schematic drawing of the two types of sensilla in *Ips typographus* containing the A cell (large amplitude spikes) for *cis*-verbenol (cV), accompanied either by a nonresponsive (*left column*) B cell (small amplitude), or a B cell for 1,8-cineole (Ci) (*right column*). (b) Responses of both sensillum types to 1 ng cV (*upper traces*), and a binary mixture of 1 ng cV and 10 μg Ci (*lower traces*). Note the inhibition of the cV response during the response to Ci in the B cell. Black horizontal bars indicate the 0.5 s stimulation period. (c) Detailed response curves to cV and binary cV : Ci mixtures showing a Ci dose-dependent inhibition of the cV response only in sensilla that also contain the Ci cell (N = 10–12). Arrows indicate the onset of the 0.5 s stimulation period (modified from [8], with permission from the publisher).

together with another ORN type that does not respond to any odorant tested so far [19]. The Ci inhibited the cV cell only in sensilla in which the two neurons were colocalized, implying that the inhibition might be due to interactions between the ORNs. To test this hypothesis, Andersson et al. [8] recorded both types of cV sensilla (with or without the Ci cell) and tested responses to binary cV/Ci mixtures. They found that not only the spontaneous activity but also the ORN response to the lowest cV dose (1 ng) was inhibited by simultaneous stimulation with high doses of Ci (1–10 μg). This inhibition occurred only in sensilla that also contained the Ci cell (Figures 6(b)-6(c)). In addition, the response to the higher cV dose (10 ng) was more strongly inhibited in sensilla where the Ci cell was colocalized. Thus, it seems plausible that the two ORNs interact, possibly by means of passive electrical interactions [54]. However, if or to which extent the reduction in pheromone trap catches by the presence of Ci [8] can be explained by the inhibition of the cV ORN remains unknown, as the excitatory input from the two ORNs provides the means also for central integration [55]. It seems like similar inhibitory interactions between colocalized ORNs occur also in other insects [51, 56, 57], but the phenomenon has so far only been systematically addressed in bark beetles.

7. Difficulties in Comparing ORN Responses to Compounds with Different Volatility

In most SSR studies, odor stimuli are prepared based on a known amount (e.g., in nano- or microgram) of compound applied to a piece of filter paper that is positioned inside a Pasteur pipette odor cartridge. Upon stimulation, the headspace in the cartridge is blown over the insect preparation. Depending on compound, solvent, and how many times the cartridge has been used, the quantity of molecules reaching the insect can be highly variable and seriously affect the ORN response [58, 59]. Indeed, different stimulation regimes, compound doses, and solvents (mostly hexane and paraffin oil) have been used in the various bark beetle SSR studies (Table 1), making it difficult to directly compare the sensitivity and specificity of ORNs characterized in different species or studies. Furthermore, the physical parameters of the odor-delivery system also affect the integrity of an airborne odor stimulus [60], which may further increase the variability among responses.

Airborne amounts of different compounds have been measured with a photoionization detector [45]. A huge variation among compounds was observed. For the most volatile compounds, such as 2-methyl-3-buten-2-ol (Figure 5), ca 80% of the headspace in the odor cartridge was lost at the first puff, even though paraffin oil was used as solvent. Airborne amounts of heavier compounds, such as linalool, were reduced by only ca 50% after 50 reiterative stimulations. In addition, compounds that were dissolved in pentane were released at a much higher rate than compounds in paraffin [45].

The large variation between compounds, solvents, and successive stimulations could easily bias electrophysiological responses in insects. This was verified by reanalyzing the response of the 3-octanol ORN of *I. typographus* [19] to two C8-alcohols (3-octanol and 1-octen-3-ol) and two C6-alcohols (*Z*3-hexenol and 1-hexanol) using both fresh (not used) and "old" (used 10 times) stimulus pipettes [45]. The ORN response to fresh pipettes was clearly different from the response to the "old" pipettes. In particular the response to the C6-alcohols was clearly lower when old pipettes were used. In fact, the difference in response was so large that it falsely implied that recordings were made from two distinct ORN classes. Such a finding suggests that it is absolutely necessary to use very strict experimental protocols for electrophysiological recordings, and that it sometimes is required to measure airborne odor amounts, especially when compounds of different volatility are used as stimuli [45].

8. Odor Coding in Bark Beetles Compared to Other Insects

In insects in general, neurons that detect pheromone constituents have a narrow tuning. Bark beetles are no exception as the ORNs that respond to aggregation pheromone compounds are, in most cases, sensitive to only one compound. The tuning width of insect ORNs detecting plant volatiles seems to range from narrow to broad, although ORN specificity is strongly correlated to the stimulus concentration tested [18]. Most of the ORNs for plant volatiles in *I. typographus* are narrowly tuned [8]. However, some show more indiscriminate responses, such as the GLV neuron that had similar sensitivity to 1-hexanol, *E*2-hexenol, and *Z*3-hexenol. This is in contrast to the highly specific GLV neurons that have been described in, for instance, scarab beetles [50, 51], and in the Colorado potato beetle [62]. The difference may be related to the fact that these other species feed on angiosperms, which presumably requires a better resolution of angiosperm dominated volatiles (i.e., GLVs) than what is needed for a conifer specialist. Many of the bark beetle ORNs are highly selective for specific enantiomers, both in terms of pheromones and plant compounds. However, this feature is not unique for bark beetles; highly enantioselective neurons have been characterized also in other insects [63–65]. In contrast, no other insect studied so far has a comparable frequency of ORNs tuned to antiattractants as the one found in *I. typographus* [19].

The co-localization of ORNs for pheromone and plant compounds in *I. typographus* is not commonly found in insects. This special type of ORN pairing may be related to the fact that host colonization in bark beetles often involves both pheromone and plant-produced compounds [8]. In addition, the colocalized neurons for plant and pheromone compounds also interacted by inhibiting their neighboring neuron while responding [8]. It is difficult to say whether a similar interaction occurs also in other insects, since it has not been systematically addressed elsewhere. However, inhibition of the spontaneous activity of the large-spiking cell when the small-spiking cell responds seems to be a common phenomenon [35, 51, 56, 57], indicating that the same type of modulation could be present. Indirect evidence for ORN interactions was found previously in the honeybee [66]. The 18–35 ORNs that are housed within honeybee

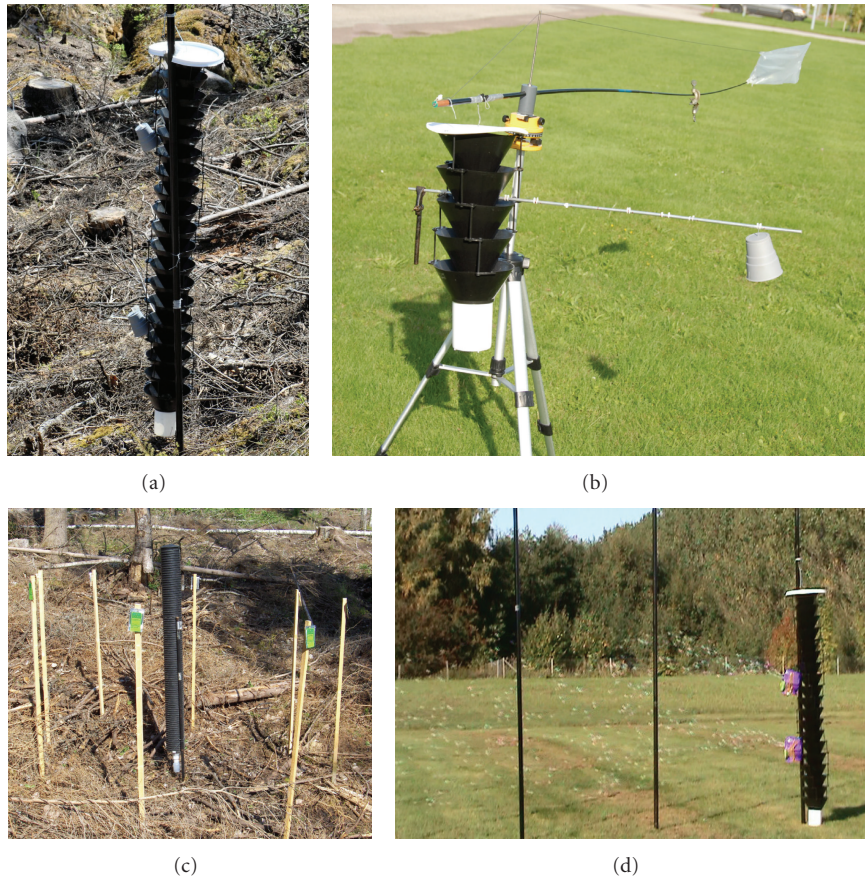


Figure 7: (a) Lindgren funnel traps (19-funnel size) were used in the vertical spacing tests with *Ips typographus*. Dispensers positioned under grey cups. (b) A Lindgren trap (5-funnel size) was attached to a wind vane in the horizontal spacing tests to ensure constant distance between plumes. (c) Pipe trap surrounded by eight nonhost volatile dispensers in the antiattractant background tests. (d) Soap bubble visualization of vertical plume overlap at a spacing distance of 48 cm. Distance between black poles = 1 m (modified from [61]).

sensilla placodea seemed to respond to odors in a coordinated manner, indicating that the individual ORNs do not act as independent response units. However, in that study, it was not possible to keep track of the individual ORNs.

Taken together, odor coding in bark beetles is, in general, similar to odor coding in other insects, but it also exhibits some rare features. The coding principle seems to be consistent with the "combinatorial code" theory, but the olfactory input travels mainly through highly specific channels.

9. Detection and Behavior in Odor-Diverse Habitats

Activation of an ORN by an attractant may cause an upwind flight by the insect towards the odor source. However, if repulsive compounds simultaneously trigger other ORNs to fire, the upwind flight may be aborted. Thus, in environments with a high "semiochemical diversity" [27] where odor plumes from different sources intermix, localization of host plants may be hampered by the presence of odors from nonhosts [67, 68]. Thus, for bark beetles, it may be possible to reduce the risk of attacks by making the environment more semiochemically diverse. Homogenous mixing of odor plumes from different sources is, however, contradicted by the partitioning of plumes into "odor packages" (or filaments) that are interspersed with pockets of "clean air" [11, 12]. This, in turn, is thought to facilitate plume discrimination by insects.

Placing an NHV mixture inside a pheromone trap, that is, next to the pheromone bait, greatly reduces trap catch of *I. typographus* [27]. However, to test the "semiochemical diversity hypothesis," pheromone trap catches in the presence of NHV at different vertical and horizontal distances from the pheromone dispenser (Figures 7(a)-7(b)), were investigated [69]. Trap catches in response to separated pheromone components (*cis*-verbenol and 2-methyl-3-buten-2-ol) were also tested (in the absence of NHV) to further investigate responses to separated baits in general. In addition, the response of the beetle was compared to the response of the Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), to separated sex pheromone components and to separated pheromone and behavioral antagonist. In both species, increased spacing between pheromone and antiattractants led to increased trap catch, whereas, as expected, increased spacing between pheromone components had the opposite effect. However,

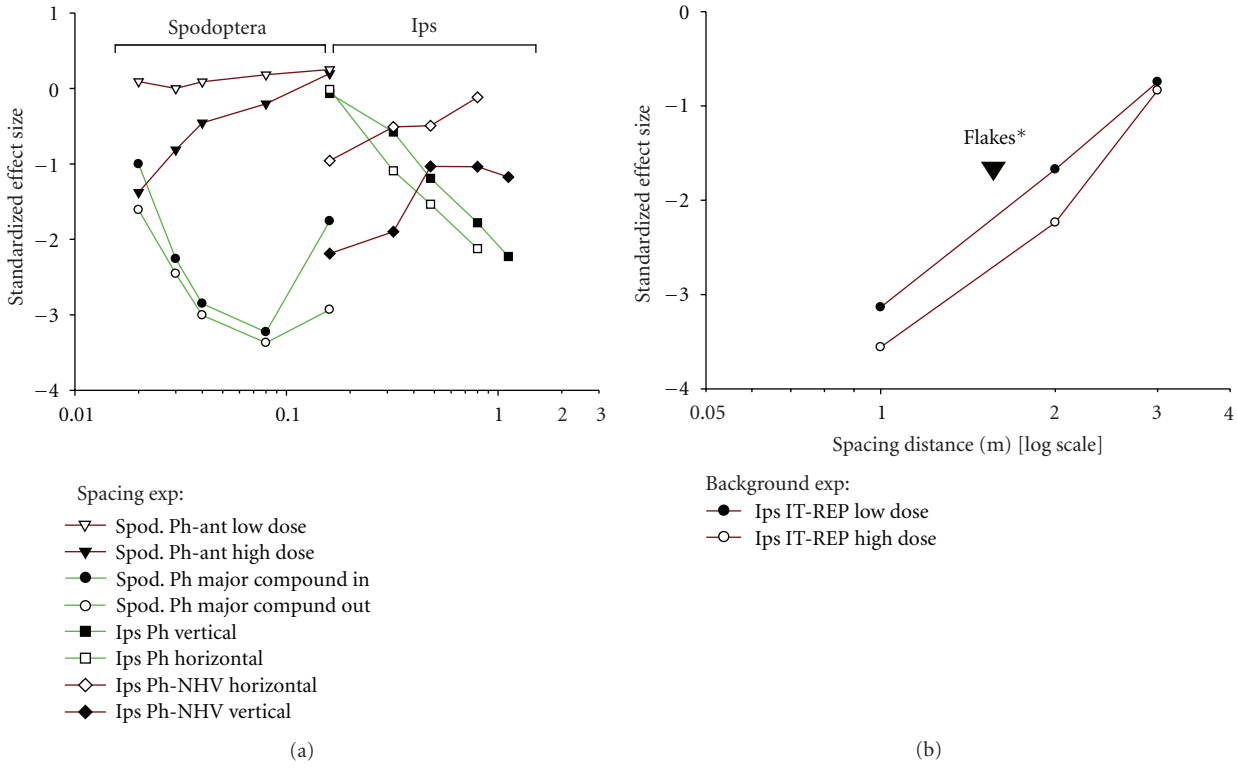


FIGURE 8: (a) The effect of spacing between attractant and antiattractant sources on trap catches of *Ips typographus* and *Spodoptera littoralis*, illustrated by measures of effect size (*Hedges*' unbiased *g*). The effect size provides a measure of a biological treatment effect by scaling the difference between the treatment and the control means, with the pooled standard deviation for those means. Effect sizes further from zero than 0.8 are regarded as strong effects. In all experiments, the pheromone bait alone (zero distance between components) was the control. The zero cm spacing distance in experiments involving antiattractants is omitted for clarity. (b) Effect sizes in the *Ips* antiattractant background experiments using nonhost volatile dispensers at eight positions, or flakes around the trap. *Flakes were evenly distributed on the ground 0–2 m from the trap. Thus, this treatment is "not to scale" on the *x*-axis. Ph = pheromone; Ant = *Spodoptera* pheromone antagonist; NHV = nonhost volatiles; IT-REP = semicommercial *Ips typographus* repellent dispenser (from [69], with permission from the publisher).

the two species differed greatly with respect to the spacing distances that affected their trap catch (Figure 8(a)). While beetle trap catches were affected by separation of some decimeters, trap catches of the moth were affected by separation distances of just a few centimeters [69]. In each species, the spacing distances affecting trap catch did not differ between the pheromone component spacing and the pheromone/antiattractant spacing experiments [69].

The bark beetle pheromone/NHV spacing experiments indicated antiattractive effects of NHV up to a distance of >1 m [69]. To further investigate potential effects of NHV at even longer distances, pheromone attraction was studied in the presence of a synthetic background of NHV, either created by eight NHV point sources positioned in a ring (with 1, 2, or 3 m radius) around a central pheromone trap (Figure 7(c)), or by ca 6000 small (ca 3 × 3 mm) NHV impregnated flakes [70] on the ground around a pheromone trap [69]. With the eight NHV sources, bark beetle attraction was reduced up to the 2 m spacing distance, and there was still a tendency for reduced attraction at the 3 m distance (Figure 8(b)). Similar to the eight point sources, the NHV flakes also reduced pheromone attraction [69]. The active spacing distances are in accordance with the "active inhibitory range" of NHV of at least 2 m that was estimated previously [27]. The pheromone dose used by Andersson et al. [69] was comparable to that released from a mass-attacked tree, which is a very strong signal. Thus, it is striking that volatiles from nonhost plants can inhibit attraction when they are released a few meters away from the pheromone source. This indicates that avoiding not only nonhost species, but also nonhost habitats, likely improves bark beetle fitness.

The different spacing distances that affected trap catches of the beetle and the moth may reflect differences in the size of the natural odor sources (and plumes) the insects orient to [69]. While a male moth orients towards a single calling female, bark beetles may orient to large patches of trees with hundreds of calling males. Furthermore, the moth sex pheromone communication system is highly specialized. A male moth flies towards a calling female for mating only, whereas the bark beetle aggregation pheromone can be used as a signal of mates, food, and oviposition sites. Thus, the different selection pressures that operate on these systems have likely resulted in different degrees of specialization. The ORNs for pheromone compounds in moths are housed in specific sensilla (*trichodea*), distinct from the ones that

detect plant odors [43]. In contrast, *I. typographus* groups the *cis*-verbenol pheromone ORN together in the same sensilla as the ORN for the plant compound 1,8-cineole, although the ORNs themselves are specific in their response [8].

Similar to *I. typographus*, studies on *Dendroctonus* bark beetles indicated synergistic interactions between pheromone components when two baits were separated by several meters [71, 72]. The sharp response of *S. littoralis* to odor source spacing has been observed previously in other moths [73–75]. The most extreme example is provided by Fadamiro et al. [52], who found that 1 mm separation between pheromone and antagonist was sufficient to restore upwind flight to the pheromone by male *Helicoverpa zea*. It was hypothesized that coincident detection of pheromone components and antagonists, achieved by colocalization of the ORNs, was the reason for this amazing ability of the males. Furthermore, synchronous detection of pheromone compounds was shown to improve the temporal spiking pattern by projection neurons in the antennal lobe of *Manduca sexta* moths [76]. Thus, it is clear that coincidence detection is of great importance in the pheromone system of moths. Soap bubble generators were used to visualize plume overlap at the different spacing distances used for *I. typographus* (Figure 7(d)) [69]. The simulations indicated that filaments from different plumes are more likely to overlap and, thus, to be detected coincidently, when the sources are close to each other. Therefore, the lower sensitivity of *I. typographus* to small-scale spatial separation of odor sources might indicate that coincidence detection is of less importance for bark beetles than for moths [69].

10. Applications

Conifer pest insect infestations are typically less common in diversified habitats [67], which in part may be due to the presence of antiattractive NHV. The finding that NHV, from a distance of at least 2 m (see also [27]), can reduce attraction to a pheromone dose comparable to that released from a mass-attacked tree suggests a potential for NHV in forest protection. However, pheromone attraction was not completely shut down so it is more likely that, instead of counteracting ongoing mass attacks, synthetic or natural NHV sources may reduce the risk of spruces being attacked in the first place. Indeed, spruces were previously protected by NHV dispensers attached to every second tree, demonstrating a protective effect of ca 2 m [77]. In another study, groups of ten trees were all protected by 20 NHV dispensers, and bark beetle attacks were diverted to trees >15 m away [78].

In addition, the spruce compound 1,8-cineole that strongly reduced pheromone attraction should be further tested in combination with the other active semiochemicals for possible improvement of antiattractant blends. It is possible that the repression of the ORN for *cis*-verbenol by 1,8-cineole, adds another inhibitory mechanism by distorting the "perceived blend ratio" of the aggregation pheromone. If so, it is likely that a more effective antiattractant blend can be obtained than the one that is comprised of GLV alcohols, C8-alcohols, *trans*-conophthorin, and verbenone [27].

11. Conclusions and Future Directions

The recent advances in bark beetle olfactory physiology have provided a connection between the physiological and behavioral responses of *I. typographus* to ecologically relevant compounds. This connection has allowed for a deeper understanding about how bark beetles (and possibly insects in general) may encode, and respond to, the odor environment. However, there are still several ORNs of *I. typographus* (and other species) for which odor ligands have not been identified, meaning that there is yet more to be learned about its olfactory physiology. Identification of active compounds should be achieved by GC-coupled SSR and by testing headspace collections from, for instance, attacked and unattacked or resistant host trees.

At the molecular level, Andersson and collaborators [61, 79] recently sequenced the antennal transcriptome of *I. typographus*, leading to identification of gene sequences for 40 different candidate olfactory receptors (ORs). The amino acid sequences of the receptors were compared, in a sequence similarity tree, with receptors that were previously identified from the genome of the flour beetle, *Tribolium castaneum*. Many of the *Ips* ORs formed a bark beetle-specific branch, indicating an extension of OR function. Possibly, these receptors detect conifer-related volatiles or pheromones that are especially relevant for bark beetles. The other ORs of *Ips* were grouped together with ORs of *T. castaneum*, which may indicate conserved functionality of some sets of ORs within Coleoptera. Functional studies to reveal which compounds the ORs of *Ips* bind will be the next step in the study. Such studies will hopefully extend the connection from behavior, through physiology, all the way to the level of the receptor and gene.

The identification of the bark beetle ORs paves the way for the development of potential novel management strategies in the future. If the receptors for pheromone components and antiattractive NHV can be identified, it might be possible to identify ligands that pharmacologically block the pheromone receptors or hyperstimulate [80] receptors for nonhost volatiles. If such compounds are found, they might be dispensed in the forest to disrupt bark beetle pheromone communication and host tree localization.

One hypothesis why insect colocalize specific ORNs in the same sensilla is that it allows for improved spatiotemporal resolution of odor stimuli [52]. This hypothesis could be tested by comparing trap catches of *I. typographus* in response to spacing between pheromone and 1,8-cineole (ORNs for *cis*-verbenol and 1,8-cineole co-localized), with trap catches in response to spacing between pheromone and verbenone, the latter compound being detected by an ORN that is never colocalized with an aggregation pheromone neuron. Predictably, the beetle should be more "sensitive" to small-scale spacing between pheromone and 1,8-cineole than to spacing between pheromone and verbenone.

In order to put the sensory physiology into a more natural context, a portable single sensillum recording device [81] should be used in the field. The sensillum that contains the ORNs for *cis*-verbenol and 1,8-cineole could be used as a biological detector for measurements of plume filament

overlap. Such measurements would reveal whether filaments from overlapping plumes are detected coincidently or not. It would also provide some indirect clue if beetles temporally integrate filaments from different plumes to a larger degree than moths, which could explain the difference in response to spacing in the two types of insects.

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