



# Verbenone—the universal bark beetle repellent? Its origin, effects, and ecological roles

Tobias Frühbrodt<sup>1</sup> · Martin Schebeck<sup>2</sup> · Martin N. Andersson<sup>3</sup> · Gerrit Holighaus<sup>4</sup> · Jürgen Kreuzwieser<sup>5</sup> · Tim Burzlaff<sup>6</sup> · Horst Delb<sup>1</sup> · Peter H. W. Biedermann<sup>6</sup>

Received: 12 October 2022 / Revised: 2 May 2023 / Accepted: 5 May 2023 / Published online: 31 May 2023  
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## Abstract

Bark beetles (Curculionidae: Scolytinae) spend most of their life in tissues of host plants, with several species representing economically relevant pests. Their behaviour is largely guided by complex olfactory cues. The compound verbenone was discovered early in the history of bark beetle pheromone research and is now sometimes referred to as a ‘universal bark beetle repellent’. However, some studies aiming to protect trees with verbenone have failed. In fact, most research effort has gone into applied studies, leaving many questions regarding the ecological functions of verbenone for various species unanswered. Here, we review and analyse the scientific literature from more than 50 years. Behavioural responses to verbenone are common among pest bark beetles (< 1% of scolytine species studied so far). Indeed, attraction is inhibited in 38 species from 16 genera, while some secondary species are unaffected or even attracted to verbenone. It is not clear whether the beetles can control the biosynthesis of verbenone; its release may not be an active signal by the beetles, but a passive cue resulting from microorganisms during host colonisation. In this context, we advocate to recognise a bark beetle and its microbiome as an entity (‘holobiont’), to better understand temporal release patterns and deduce the specific function of verbenone for a given species. Surprisingly, natural enemies are not commonly attracted by verbenone, but more taxa need to be studied. A better understanding of the ecological functions of verbenone will help to make verbenone-based tools more effective and improve integrated pest management strategies.

**Keywords** Anti-aggregation · Bark beetle management · Chemoecology · Natural enemies · Scolytinae

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Communicated by Antonio Biondi.

✉ Tobias Frühbrodt  
Tobias.Fruhbrodt@forst.bwl.de

<sup>1</sup> Department of Forest Protection, Forest Research Institute Baden-Württemberg, Freiburg, Germany

<sup>2</sup> Department of Forest and Soil Sciences, Institute of Forest Entomology, Forest Pathology and Forest Protection, University of Natural Resources and Life Sciences Vienna, BOKU, Vienna, Austria

<sup>3</sup> Department of Biology, Lund University, Lund, Sweden

<sup>4</sup> Department of Forest Protection, Northwest German Forest Research Institute, Göttingen, Germany

<sup>5</sup> Chair of Ecosystem Physiology, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg, Germany

<sup>6</sup> Chair for Forest Entomology and Protection, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg, Germany

## Key message

- Verbenone is long known and now sometimes referred to as a ‘universal bark beetle repellent’.
- Research focus has mostly gone into applied studies, leaving many ecological questions unanswered.
- Here, we review the scientific literature from more than 50 years to interpret present knowledge.
- The function of verbenone as an active signal or passive cue may vary across bark beetle species.
- Filling identified gaps of knowledge will improve verbenone-based pest management approaches.

## Introduction

Bark and ambrosia beetles of the weevil subfamily Scolytinae are exceptional as they spend almost their entire life inside the protective tissues of various plants. Thus, some of them are, or have the potential, to become economically relevant pests in agricultural and forest ecosystems, even though the great majority are harmless to living plants (Gregoire and Evans 2004; Raffa et al. 2015). During the short phase of adult dispersal, they have to find and colonise a host tree, and encounter mating partners. This critical period of their life cycles is characterised by high mortality rates due to natural enemies and abiotic stressors (Lindgren and Raffa 2013). Thus, there is a strong selection on efficient recognition for both suitable host plants and mates. Host and mate searching behaviours, as well as host choice, are regulated by complex interactions of different environmental cues, which may be visual, gustatory, and olfactory, originating from conspecifics and heterospecifics of various trophic levels (Byers 1989, 2004; Borden 1997; Strom et al. 1999). Semiochemicals are olfactory cues that can be grouped by their origin and their implications for the sender and the receiver (Nordlund and Lewis 1976). Bark beetles rely on a broad range of semiochemicals (Borden 1997), including pheromones that are released and received by individuals from the same species, and allelochemicals that mediate communication between species (Nordlund and Lewis 1976). The latter are further divided into kairomones that are released by one species (e.g. host trees) and are to the benefit of the receiver of another species (e.g. bark beetles), allomones that are beneficial for the emitter of another species, and synomones that are to the benefit of both the sender and the receiver species (Nordlund and Lewis 1976).

Bark beetles deploy different strategies to interact with their hosts. They can be classified according to whether they colonise dead or dying trees (hereon referred to as ‘secondary’ species), they live in vital trees as parasites or their colonisation results in the death of their host trees (hereon referred to as ‘primary’ species) (Lindgren and Raffa 2013). Additionally, bark beetles can be gregarious—i.e. many individuals aggregate to colonise a tree by the attraction of male and female conspecifics—or solitary (Raffa et al. 2015). Following host acceptance, gregarious bark beetle species start to release aggregation pheromones to attract conspecifics for mating and to collectively overcome tree defence (Silverstein et al. 1966; Raffa and Berryman 1983; Berryman et al. 1985). There is a large variety of mating systems in bark beetles, e.g. in respect to the sex that initiates the gallery or the number of females per male in polygynous mating systems (Kirkendall 1983).

Gregarious bark beetle species risk to suffer intraspecific competition from too many galleries inside a tree

that will reduce the survival odds of the offspring and thus decrease fitness (Anderbrant et al. 1985; Sallé and Raffa 2007). Two non-mutually exclusive mechanisms have been proposed to terminate the colonisation of a tree: the cessation of the synthesis of aggregation pheromone components, and the release of anti-aggregation pheromones (Byers and Wood 1980; Byers et al. 1984; Vité and Francke 1985; Pureswaran et al. 2000). Most scolytine species are non-gregarious, including solitary bark beetles without known aggregation pheromones, as well as most of the paraphyletic group of ambrosia beetles, cultivating fungi in the wood as a food source (Lindgren and Raffa 2013; Hulcr and Stelinski 2017). In non-gregarious and secondary species, the effect of interaction with conspecifics is even more disadvantageous because there is no gain from collective colonisation, instead all conspecifics impose immediate competition for limited resources (Raffa 2001), which means that also these species presumably have an advantage in detecting and reacting to non-suitable, possibly already colonised, host trees.

In the context of avoidance of non-suitable hosts, verbenone has been discovered in bark beetles more than half a century ago (Renwick 1967). It was early proposed as an anti-aggregation component (Renwick and Vité 1969; Rudinsky 1973). Libbey et al. (1974) and Rudinsky et al. (1974) were the first to use the term ‘anti-aggregative pheromone’ with the purpose of terminating attacks (Byers et al. 1984; Leufvén et al. 1984; Byers 1989; Hunt and Borden 1990) and shift new attacks to adjacent trees (Bakke 1981). Because verbenone is also associated with the decay of wood infested by bark beetles (Byers et al. 1989), it is also referred to as an indicator of old and thus unsuitable host material (Lindgren and Miller 2002b; Byers 2004). Indeed, much of the verbenone biosynthesis may actually be derived from microbes inside or outside the beetles’ bodies and the respective contributions of insect vs. microbes to verbenone biosynthesis are still elusive (Byers et al. 1984, 1989; Leufvén and Birgersson 1987; Hunt and Borden 1990). In some bark species, verbenone may thus be an actual ‘signal’, that is purposefully released by the sender—and would thus be called a pheromone—while in other species, it may be a passive ‘cue’ emitted by microorganisms (Leonhardt et al. 2016).

Today, the concept of verbenone as a ‘universal bark beetle repellent’ (Audley et al. 2020; Huber et al. 2020) or a ‘general sign of host unsuitability’ (Byers 2004) is well established and is, besides non-host or green leaf volatiles (Byers et al. 1998, 2004; Zhang and Schlyter 2004), one of the most prominent bark beetle aggregation inhibitors (Mafra-Neto et al. 2022). Numerous studies focussed on the potential of verbenone in pest population management as a repellent tree protectant (e.g. Richerson and Payne 1979; Bedard et al. 1980; Livingston 1983; Borden et al.

1997). However, this has impeded the further investigation of its ecological function and underlying response mechanisms (Borden 1997). Some studies also failed to demonstrate the inhibitory effect of verbenone (e.g. Jakuš et al. 2003), leading some authors to speak of ‘a history of unpredictability’ (Progar 2003). The major reason is that we still know little about the reasons why verbenone seems to be a ubiquitous cue in many bark beetle species, while applied studies observe so much uncertainty. There are still many open questions on the ecological and evolutionary roles of verbenone for the highly diverse group of bark beetles with various life histories. Is it an actual anti-aggregation pheromone synthesised by the beetles themselves or rather an indicator of poor host quality (degradation or competition)? Does it only inhibit attraction under the presence of aggregation pheromones to mask a fully colonised host or is verbenone repellent itself also in the absence of attractive compounds? Does it mediate intraspecific and also interspecific competition for breeding habitat and food resources? And finally, if verbenone is so commonly associated with bark beetles, are higher trophic levels such as predators and parasitoids also able to rely on verbenone to detect their prey?

Here, we summarise and synthesise a broad body of literature from more than 50 years of basic and applied research on the effects of verbenone on bark and ambrosia beetles. We focus on pheromone biosynthesis and perception and infer its ecological implications in the communication of these insects. A simple quantitative meta-analysis was conducted on a few species for which the response to verbenone has been studied, to relate a species’

response to verbenone with its mating system and its host use behaviour (primary vs. secondary species).

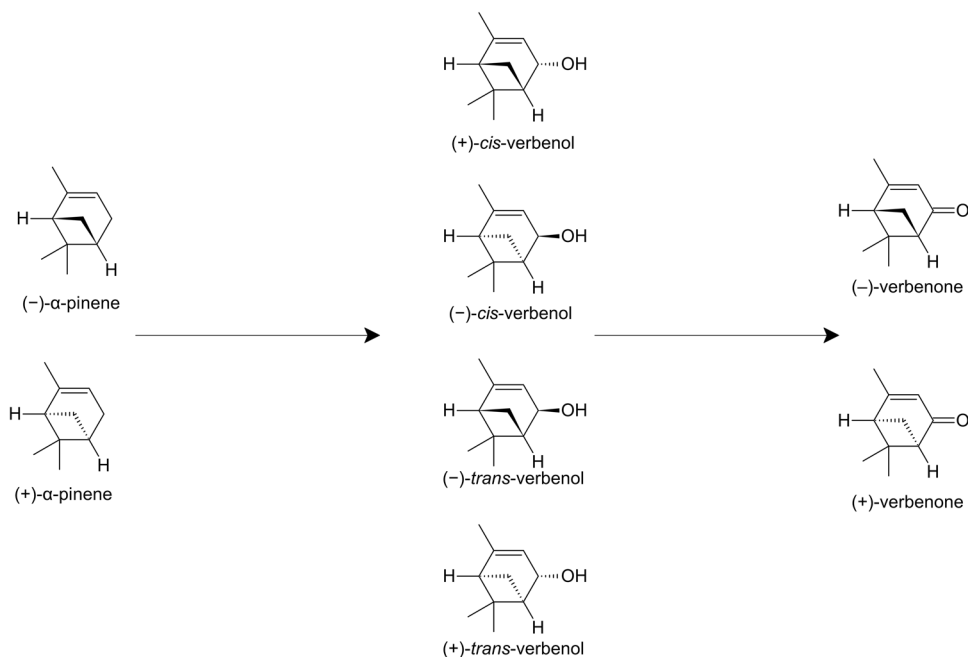
In the following, we first focus on the emission of verbenone in space and time. Q1.1.: Who and where?—Which organisms produce verbenone? Which anatomical structures are involved in biosynthesis? What are the relevant pathways? Q1.2.: When and how much?—In which phases of host colonisation is verbenone produced? Which quantities are ecologically relevant? The second part then focusses on the receivers of verbenone as a behaviourally relevant cue. Q2.1: Olfaction and neurophysiology—How is verbenone detected? Q2.2: Behaviour—What is the behavioural response of bark beetles to verbenone, and which factors influence this response? Finally, the role of verbenone is set into a larger ecological and evolutionary context discussing its implications on intra- and interspecific interactions, as well as trophic relationships.

## The roles of verbenone in the natural history of bark beetles—an overview

### Verbenone emittance—Who is producing it?

Verbenone, i.e. 4,6,6-trimethyl-bicyclo[3.1.1]hept-3-en-2-one or 2-pinen-4-one (CAS 80–57-9, Fig. 1), is a monoterpene ketone with two enantiomers: (–)-*S*- and (+)-*R*-verbenone. It was first identified as an oxidation product in coniferous turpentine oil (Blumann and Zeitschel 1913). Accordingly, it can be found in various gymnosperm tree species (Shepherd et al. 2007), including common bark

**Fig. 1** Chemical synthesis of verbenone from its pre-precursor  $\alpha$ -pinene and its precursor verbenol with all existent isomers



beetle host species (Flechtmann et al. 1999; Dvořáková et al. 2007; Liazid et al. 2007; Szmigielski et al. 2012), but also in various angiosperm species (e.g. Shepherd and Sullivan 2013; Guo et al. 2017; Pedrali et al. 2019; Çetin and Güdek 2020).

### Q1.1: Who and where?—Which organisms produce verbenone?

#### Chemical synthesis

Verbenone synthesis almost inevitably occurs under an aerobic environment when one or both of the two precursors  $\alpha$ -pinene, a major component of conifer resin (Lieutier et al. 1989; Nagel et al. 2022) or verbenol, an oxidation product of the former, are present (Blomquist et al. 2010; Ramakrishnan et al. 2022; Fig. 1). The conversion of verbenol to verbenone can occur spontaneously by autoxidation (Bhattacharyya et al. 1960), but this only seems to matter after long time spans: the percentage of verbenone in turpentine oil from *Picea abies* increased from initially 0.2% to 50% after 20 days (Dvořáková et al. 2007), even though this can (Flechtmann et al. 1999), but does not necessarily lead to an increase in verbenone release from cut logs after a comparable time span (Pettersson and Boland 2003). The oxidation of verbenol into verbenone is irreversible (Leufvén et al. 1984; Lindmark-Henriksson et al. 2003) and without by-products (Brand et al. 1976).

Apart from autoxidation, the beetles themselves or associated microorganisms enhance verbenone production. These include the beetle-associated ascomycete blue stain fungi (Cale et al. 2019; Kandasamy et al. 2023), yeasts (Brand et al. 1976; Leufvén et al. 1984; Hunt and Borden 1990) and bacteria (Xu et al. 2015). Also, wood-degrading basidiomycetes have been shown to biotransform  $\alpha$ -pinene to verbenol (Busmann and Berger 1994; Trapp et al. 2018), as well as saprophytes (Bhattacharyya et al. 1960; Agrawal et al. 1999). The capability to convert verbenol to verbenone seems to be common among bark beetle-associated microorganisms: Leufvén et al. (1984) found six yeast species from the guts of *Ips typographus*, capable of converting *cis*-verbenol to verbenone. In fact, microorganisms might not only be capable of biosynthesising verbenone, they might even be the main responsible agents. For example, in *Dendroctonus valens* fed with antibiotics, the verbenone amounts in guts and frass were considerably lower in both sexes compared to the antibiotic-free control group (Xu et al. 2015), but it is not clear whether the remaining amounts were due to autoxidation or the beetles' metabolism. Also, numerous bacterial isolates were capable of converting *cis*-verbenol to verbenone, including *Pseudomonas* spp., *Serratia* sp. and *Rahnella aquatilis*, which also made a high proportion of the bacterial community in the gut and frass (Xu et al.

2015). Similarly, in *Dendroctonus ponderosae* suppression of associated microorganisms even completely inhibited the synthesis of verbenone (Hunt and Borden 1989), suggesting that the beetles alone do not biosynthesise verbenone. These examples demonstrate the potential importance of microorganisms in verbenone biosynthesis; however, it is still less clear, to what extent they contribute under natural conditions (Hunt and Borden 1990) and whether autoxidation could also be relevant. In conclusion, it seems that bark beetles accelerate the inevitable decay of host tissue by introducing microbes, resulting in the release of verbenone. However, to our knowledge, no enzymes that mediate the conversion of verbenol to verbenone have been characterised so far, so that the underlying biochemical processes are not known.

#### Stereochemistry

Verbenone, its direct precursors verbenol and its pre-precursor  $\alpha$ -pinene are chiral compounds (Fig. 1). The intermediate product verbenol also possesses two geometric isomers (*cis*- and *trans*-verbenol). In many studies, the optical isomers of verbenone originating from bark beetles are not further differentiated (e.g. Shepherd et al. 2007; Zhang et al. 2007a, b; Shi and Sun 2010; Liu et al. 2019; Ramakrishnan et al. 2022) and are thus comparatively poorly assessed.

It seems that (–)-verbenone might be the more relevant enantiomer in bark beetle chemical ecology. It was predominant in hindgut extracts from male *Pseudips orientalis* (Zhang et al. 2011), *Ips nitidus* (Zhang et al. 2009) as well as *Ips pini* (Pureswaran et al. 2000), and it was the only conversion product from *trans*-verbenol of two ophiostomatoid fungi associated with *D. ponderosae* (Cale et al. 2019). The enantiomeric configuration of verbenone is determined by its pre-precursor  $\alpha$ -pinene (*R*-, *S*-, or racemic) (Lindmark-Henriksson et al. 2003; Fang et al. 2020). Concordantly, verbenone biosynthesis may not be enantioselective within a species but depend on the host substrate. As a consequence, also the resulting ratios of both verbenone enantiomers are variable within a species. For example, in *D. frontalis* ratios of (+)- and (–)-verbenone varied strongly (between 20 and 80%) among sexes and across geographic regions for males (Grosman et al. 1997), probably due to variation in enantiomeric ratios of  $\alpha$ -pinene (Lindström et al. 1989; Taft et al. 2015). A rather strong intraspecific variation in the ratio of both enantiomers is consistent with the finding that typically both enantiomers elicit a behavioural response to some degree (e.g. Byers et al. 2018, see Q2.1; but see Dickens 1979; Raffa 2001; Zhang et al. 2006).

The geometric configuration of the intermediate substrate verbenol also seems to be important. Both enantiomers of verbenol can lead to the synthesis of verbenone. *cis*-Verbenol can also be converted to *trans*-verbenol (Leufvén et al. 1984; Hunt and Borden 1990; Lindmark-Henriksson

et al. 2003), but the reverse reaction has not been reported. This one-way reaction might represent a ‘dead end’ and impede verbenone synthesis, e.g. only three of six yeast strains extracted from the guts of *I. typographus* were able to produce verbenone from *trans*-verbenol, but all six were able to do so when *cis*-verbenol was the substrate (Leufvén et al. 1984). In two *Dendroctonus* species *trans*-verbenol was the predominant isomer: both sexes of *D. brevicomis* were capable of converting either enantiomer of  $\alpha$ -pinene to the respective enantiomer of *trans*-verbenol and myrtenol with only trace amounts of *cis*-verbenol (Byers 1983a, b; Byers et al. 1984). Similarly, two ophiostomatoid fungi typically associated with *D. ponderosae* exclusively produced (–)-verbenone from *trans*-verbenol (Cale et al. 2019). The fate of *cis*-verbenol to be converted directly to verbenone or to *trans*-verbenol instead also depended on the optical configuration of *cis*-verbenol for at least some of the yeasts extracted from *I. typographus* (Leufvén et al. 1984). Probably due to these differences in the synthesis, typically one of the two isomers of verbenol predominates in a given species. Interestingly, the respective other enantiomer can typically be found to a smaller extent, as is the case in *I. typographus* (Birgersson et al. 1984; Leufvén and Birgersson 1987; Schlyter et al. 1987; Birgersson and Bergström 1989; Ramakrishnan et al. 2022), *D. frontalis*, and *D. ponderosae* (Grosman et al. 1997; Pureswaran et al. 2000).

### Site of verbenone biosynthesis

Unlike other insect species where pheromones are biosynthesised in specific cells or glands located in the abdomen and/or epidermal cells (Tillman et al. 1999), bark beetles do not have specific organs for pheromone biosynthesis (Tittiger and Blomquist 2016). Instead, evidence from many species suggests that the major organ of verbenone synthesis is the hindgut (Table 1), as is also described for most bark beetle pheromones (Byers 1989), particularly those derived from host monoterpenes (Tittiger and Blomquist 2016). This is coherent with the finding above that verbenone biosynthesis is mostly mediated by microorganisms, which are located in the gut.

The relative contribution of the gut to verbenone emission from bark beetle infestations, however, is not yet clear. Verbenone amounts increased in headspace samples of Scots pine logs infested with *Tomicus piniperda* over 5 days, while non-infested control logs released little verbenone at a constant rate (Byers et al. 1989), stressing the importance of bark beetle colonisation for verbenone emission. Other studies likewise detected verbenone outside the beetles’ bodies. For instance, bacteria associated with *D. valens*, like *Pantoea conspiciua*, *Enterobacter xiangfangensis*, *Staphylococcus warneri* and at least six other species, were responsible for the conversion of verbenol to verbenone in adult frass

(Xu et al. 2015, 2016; Cao et al. 2018). Leufvén and Birgersson (1987) analysed the chemical composition of the phloem around galleries of *I. typographus* at different attack phases on Norway spruce. They proposed four possible pathways explaining the occurrence of oxygenated monoterpenes—including verbenone—in the phloem, which could (i) either be released by the beetles and thereafter absorbed by the phloem, (ii) synthesised by associated microorganisms, (iii) oxygenated via host-derived enzymes, or (iv) via autoxidation (Leufvén and Birgersson 1987). Thus, a substantial amount of verbenone could be produced in close proximity outside the beetles; however, the relative importance of each source is still not clear.

### Sex-specific differences

Across many species with different mating systems, males seem to produce more verbenone compared to females. In *Dendroctonus* species, females are the gallery-initiating sex. In female *D. brevicomis*, no verbenone was detected, while considerable amounts were found in males (Byers 1983a). This was also the case for *D. ponderosae*, where a switch in verbenone synthesis from females to males was proposed upon mating (Pureswaran and Borden 2003). The same was proposed for *D. frontalis* (Rudinsky 1973), which is in conflict with the finding of another study that found that hindgut extracts from male beetles had 68-fold greater amounts of verbenone compared to females already right after emergence (Grosman et al. 1997). In agreement, solvent extraction and chemical analysis of male and female *D. brevicomis* that had recently landed on a ponderosa pine showed that females lacked verbenone and males had a high amount of verbenone, which declined after joining a female in her gallery (Byers and Wood 1980; Byers et al. 1984). Regardless of the time point, males in *Dendroctonus* seem to have greater amounts of verbenone. Interestingly, in Grosman et al. (1997) the females were found to accumulate *trans*-verbenol, which might be further oxidised to verbenone later on. An accumulation of *trans*-verbenol in females was also reported for the monogynous *Conophthorus coniperda*, while males contained verbenone (Birgersson et al. 1995). It is not clear whether the verbenol-to-verbenone ratio also changes in the females of these species during colonisation or if *trans*-verbenol is directly released without further oxygenation. Also in the monogynous species *T. piniperda*, males contained more verbenone than females (Lanne et al. 1987), while this was the opposite in *Tomicus minor* (Wu et al. 2019). In *Ips* spp. and other polygynous species, males initiate the galleries and are typically the main aggregation pheromone producers (Byers 1989). Accordingly, almost no aggregation pheromone components were found in female *I. typographus* (Birgersson et al. 1984), *I. nitidus* (Zhang et al.

**Table 1** Mean or range of absolute amounts of verbenone reported for individual bark beetle species across various phases of colonisation

| Species                         | Time point/phases   | Origin/method   | Amount (♂, ♀) of verbenone (range or mean)               | References   |
|---------------------------------|---|---|--|--|
| <i>Dendroctonus armandi</i>     | Unmated ♀ in nuptial chamber, 1♀ + 1♂, 1♀ + 2♂            | Hindgut extracts, beetles from natural windthrows   | ♀: 36–73 ng/beetle                                       | Xie and Lv (2012)                                      |
| <i>Dendroctonus brevicomis</i>  | After 18 h exposure to α-pinene and right after emergence | Hindgut extracts, beetles from laboratory rearing   | ♂: 620–2800 ng/beetle<br>♀: no verbenone detected        | Byers (1983a)  |
|                                 | After 18 h exposure to α-pinene and right after emergence | Hindgut extracts, beetles from laboratory rearing   | adult ♂: 2300–2800 ng/beetle<br>♀: no verbenone detected | Byers (1983b)  |
|                                 | Daily for 13 days   | Hindgut extracts, beetles from induced infestation on standing tree   | ♂: 0–1600 ng/beetle<br>♀: no verbenone detected          | Byers et al. (1984)                                    |
| <i>Dendroctonus frontalis</i>   | Unmated ♀, mated beetles, oviposition                     | Headspace sampling from individual boreholes on natural attack site   | 140–300 ng/2 h   | Pureswaran and Sullivan (2012)                         |
|                                 | After emergence   | Hindgut extracts, beetles from natural infestation emerged under laboratory conditions  | ♂: 3300–7900 ng/beetle<br>♀: 30–120 ng/beetle            | Grosman et al. (1997)                                  |
| <i>Dendroctonus ponderosae</i>  | 12–120 h  | Headspace samplings from individual boreholes after artificial inoculation  | 0–400 ng/mL (in 4 h)                                     | Cale et al. (2015)                                     |
|                                 | 12–108 h  | Headspace samplings from individual boreholes after artificial inoculation  | 0–250 ng/mL  | Taft et al. (2015)                                     |
|                                 | Newly emerged, unmated, mated                             | Beetle extracts, beetles from naturally infested logs emerged under laboratory conditions   | ♂ + ♀: 0–66 ng/beetle                                    | Pureswaran and Borden (2003), Pureswaran et al. (2000) |
|                                 | Newly emerged, unmated, mated                             | Headspace sampling from grouped beetles in the laboratory, beetles from naturally infested logs emerged under laboratory conditions | ♂: 3–5 ng/beetle<br>♀: 10–40 ng/beetle                   | Pureswaran et al. (2000)                               |
| <i>Dendroctonus rhizophagus</i> | No feeding; after 24 h of feeding, unmated and mated      | Hindgut extracts (passive), beetles from inoculation on logs in the laboratory  | ♂: 27–54 ng/beetle<br>♀: 17–107 ng/beetle                | Cano-Ramírez et al. (2012)                             |
|                                 | Unmated, mated, oviposition, after male re-emergence      | Hindgut extracts (passive), beetles from natural infestation site   | ♂: 0–150 ng/beetle<br>♀: 75–300 ng/beetle                | Cano-Ramírez et al. (2012)                             |
| <i>Dendroctonus valens</i>      | Newly emerged, unmated, mated                             | Hindgut extracts, beetles from baited traps in the field  | ♂: 5–31 ng/beetle<br>♀: 1–27 ng/beetle                   | Shi and Sun (2010)                                     |
|                                 | After 96 h of feeding                                     | Gut extracts, beetles from pine phloem medium   | ♂: 31 ng/beetle<br>♀: 71 ng/beetle                       | Xu et al. (2015)                                       |
| <i>Ips amitinus</i>             | NA  | Hindgut extracts, beetle origin not clear   | ♂: verbenone as minor component                          | Zuber (1994)   |
| <i>Ips cembrae</i>              | 6 phases  | Hindgut extracts, beetles from natural infestation  | ♂ + ♀: <5 ng/beetle                                      | Zhang et al. (2000a, b)                                |
| <i>Ips nitidus</i>              | Unmated, mated ♂ with 1, 2 and 3 ♀                        | Hindgut extracts, beetles from natural infestation  | ♂: 20–84 ng/beetle<br>♀: no verbenone detected           | Zhang et al. (2009)                                    |

**Table 1** (continued)

| Species                     | Time point/phases                             | Origin/method   | Amount (♂, ♀) of verbenone (range or mean)   | References                      |
|-----------------------------|---|---|--|---------------------------------|
| <i>Ips pini</i>             | Newly emerged, unmated, mated                 | Headspace sampling from grouped beetles in the laboratory, beetles from naturally infested logs emerged under laboratory conditions | ♂ + ♀: 5–20 ng/beetle                        | Pureswaran et al. (2000)        |
| <i>Ips typographus</i>      | 0–7 days                                      | Headspace sampling from individual boreholes on natural attack site   | 0–120 ng/3 h                                 | Birgersson and Bergström (1989) |
|                             | 7 phases                                      | Hind and midgut extracts, beetles from natural infestation  | ♂: < 1 ng/beetle<br>♀: no verbenone detected | Birgersson et al. (1984)        |
|                             | 6 phases                                      | Gut extracts, beetles cultivated on logs  | ♂ + ♀: < 5 ng/beetle                         | Ramakrishnan et al. (2022)      |
|                             | Unmated, mated ♂ with 1, 2, and 3 ♀           | Hindgut extracts, beetles from natural infestation site   | ♀: 18–66 ng/beetle                           | Xie and Lv (2013)               |
| <i>Pseudips orientalis</i>  | Unmated ♂                                     | Hindgut extracts, beetles from natural windthrow  | ♂: ~ 80 ng/beetle                            | Zhang et al. (2011)             |
| <i>Tomicus brevipilosus</i> | 5 phases according to gallery length          | Hindgut extracts, beetles from natural infestation site   | ♂: 0–9 ng/beetle<br>♀: 0–10 ng/beetle        | Liu et al. (2019)               |
| <i>Tomicus minor</i>        | Recently mated                                | Hindgut extracts, beetles from windthrown trees   | ♂: ~ 1 ng/beetle<br>♀: ~ 0.4 ng/beetle       | Lanner et al. (1987)            |
|                             | Not clear if beetles were allowed to feed     | Hindgut extracts, pre-emerged beetles from natural infestation site   | ♂: 0–10 ng/beetle<br>♀: 0–13 ng/beetle       | Wu et al. (2019)                |
| <i>Tomicus piniperda</i>    | 1–140 h, unmated females or mated with males  | Headspace sampling from inoculated windthrow  | 0–240 ng/h                                   | Byers et al. (1989)             |
|                             | Recently mated and nuptial chamber terminated | Hindgut extracts, beetles from windthrown trees   | ♂: 0.7–3.8 ng/beetle<br>♀: 0.1–2.3 ng/beetle | Lanner et al. (1987)            |
| <i>Tomicus yunnanensis</i>  | Not clear if beetles were allowed to feed     | Hindgut extracts, pre-emerged beetles from natural infestation site   | ♂: < 5 ng/beetle<br>♀: < 5 ng/beetle         | Wu et al. (2019)                |

2009), *Ips duplicatus* (Zhang et al. 2007b) and *P. orientalis*, including also verbenone (Zhang et al. 2011).

As described above, the biosynthesis of verbenone is linked to the host substrate and the detoxification of  $\alpha$ -pinene. However, since both sexes usually feed on the same substrate, differences in the amounts of verbenone must be derived from differences in the downstream pathways. When the microbial conversion of *cis*-verbenol to verbenone was inhibited by antibiotics, the resulting verbenone concentration in the guts and in the frass was considerably more reduced in female *D. valens*, compared to males (Xu et al. 2015). This indicates that the relative importance of specific biosynthesis pathways may differ between the sexes, possibly by sex-specific microbial communities (see Xu et al. 2016; Whittle et al. 2021). In the guts of *I. typographus* performing maturation feeding, high amounts of verbenone were initially found in both sexes (Ramakrishnan et al. 2022). This changed after the end

of the maturation feeding and the emergence from brood trees, when verbenone was only found in males. Apparently, something in the metabolism must have changed (but this is not discussed further in the study). Similarly, male *D. brevicornis* exposed to vapours of the precursor  $\alpha$ -pinene contained verbenone in detectable amounts while females did not (Byers 1983b). For other pheromone components, it has been shown that sex-specific differences are also due to differences in gene expression (reviewed in Blomquist et al. 2010). Sex-specific differences in the amounts of pheromone components are common in bark beetles (Birgersson et al. 1995; Byers 1989). It is noteworthy that in most cases this sex bias in verbenone content is of quantitative, not qualitative nature. It remains unclear though, whether the male bias in the synthesis of verbenone in many bark beetles is due to differences in the microbiome, differences in the feeding behaviour, a mixture of both, and/or whether active regulation is possible.

## Q1.2: When and how much? In which phases of host colonisation is verbenone produced?

### Concentrations and release rates

The composition of pheromone bouquets in bark beetles is typically studied from gut extracts or by headspace sampling. Only few studies analysed the content in the phloem or frass. Relative to its precursors, the amounts of verbenone are typically low. The amount of verbenone was only around 20% of the amount of verbenol in gut extracts of *P. orientalis* (Zhang et al. 2011), only 4% in *Dendroctonus banksiana* (Cale et al. 2015), but 50% in recently mated *D. valens*, whereas this was only 6% compared to the  $\alpha$ -pinene content (Zhang and Sun 2006). These ratios from tissue extracts also seem to be reflected in the actual emission patterns. The maximum release rate of verbenone compared to the respective verbenol precursor was three times lower in *D. frontalis* and *I. typographus* (Birgersson and Bergström 1989; Pureswaran and Sullivan 2012). Most pheromones would be assumed to require short half-life spans because the information they convey is only valid for a short period of time (Shiota and Sakurai 2020). This may not be the case for verbenone as an indicator of poor habitat quality, because these conditions—once attained—are unlikely to improve again. Instead, the low availability of verbenone may be explained by its high sensitivity to sunlight under which it is photoisomerised to (+)-chrysanthenone (Kostyk et al. 1993), filifolone (Fettig et al. 2009a) and other transformation products (Erman 1967).

Absolute amounts of verbenone vary within a species depending on the host tree quality and feeding substrate (Byers 1983a; Raffa 2001; Taft et al. 2015), but also among individuals within a tree (Birgersson and Bergström 1989). Most individuals contain rather low levels, while only few individuals produce large amounts (Grosman et al. 1997; Zhang et al. 2000a, b). Absolute verbenone amounts commonly range from 0 to 100 ng per individual in a broad range of bark beetle species (Table 1). It is remarkable that in *I. typographus* maximum amounts of verbenone were less than 5 ng/beetle for both sexes across multiple life stages before and after mating (Birgersson et al. 1984; Ramakrishnan et al. 2022)—and another study even failed to detect verbenone at all (Birgersson et al. 1988)—even though verbenone is thought to be a relevant component in the pheromone communication of that species (Schlyter et al. 1989). Only low amounts of verbenone were also found in *I. amitinus* (Zuber 1994, Table 1) and *I. cembrae* (Zhang et al. 2000a, b). Contrarily, considerably larger amounts of verbenone (600–8,000 ng/beetle) were detected in male *Dendroctonus brevicomis* (Byers 1983a, b; Byers et al. 1984) and *D. frontalis* (Grosman et al. 1997). It is not clear why these two species contain up to tenfold more verbenone than

other species, even from the same genus. Larger or heavier beetles may also produce larger amounts of pheromones (Anderbrant et al. 1985; Pureswaran and Borden 2003). However, body size cannot be the main factor here because *D. frontalis* is the smallest *Dendroctonus* species (Six and Bracewell 2015), and also *D. brevicomis* has an average body length of only 3.9 mm (Valerio-Mendoza et al. 2019). In all three studies cited above, beetles have been dissected right after emergence, so that the much larger amounts of verbenone compared to other species could be in parts due to an exhaustive maturation feeding and thus be a matter of timing. In fact, samples of callow adult males and females taken from under the bark, and emerged males and females of *D. brevicomis* showed that only emerged males contained large amounts of verbenone (Byers 1983b). At least in *D. frontalis*, also the relative amount of verbenone was high: it made up to 96% of the oxygenated monoterpenes (Grosman et al. 1997), which indicates that verbenone may be of great relevance for this species. The remarkably high amounts of verbenone may possibly be derived from differences in the metabolism of *D. brevicomis* and *D. frontalis* from that of other bark beetles and could indicate an active control of the synthesis, which requires further investigation.

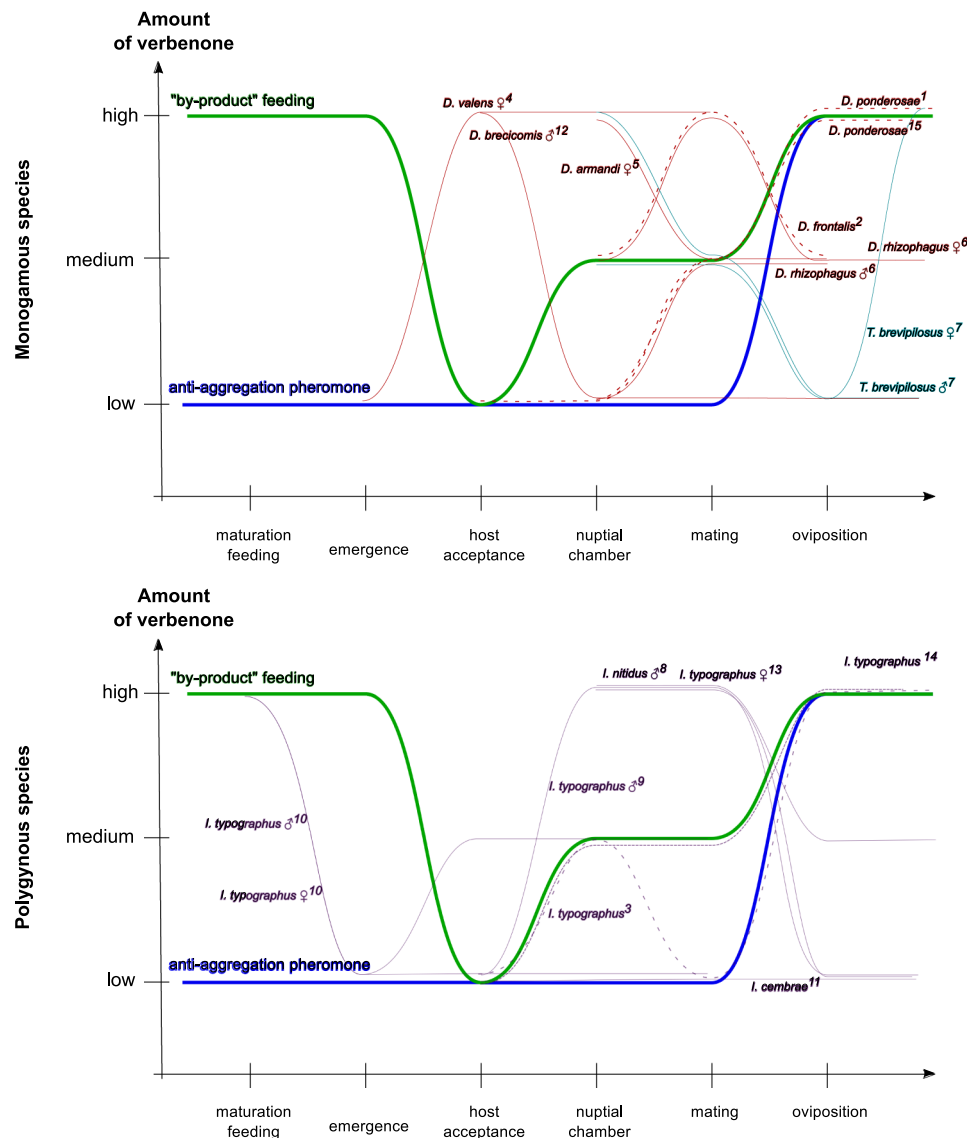
Data on atmospheric verbenone concentrations, both natural background concentrations and in the proximity of trees colonised by bark beetles, are scarce. Verbenone has been identified from conifer needle litter (Isidorov et al. 2003), and atmospheric concentrations of around 0.17–1.32 ng/m<sup>3</sup> have been found in urban, agricultural and also coniferous forest landscapes (Shimmo et al. 2004). While such concentrations are presumably below behaviourally relevant thresholds, these results can serve as a baseline to understand the natural dynamics in the release of verbenone from bark beetle infestations. The only estimation of verbenone release from an infested tree was reported in Salom et al. (1992) as unpublished data assuming that 0.45 mg/h of verbenone would be released from a tree infested with 1,400 pairs of *D. frontalis*. Headspace sampling around 50 artificial boreholes from female *T. piniperda* revealed release rates of up to 240 ng verbenone per hour (Byers et al. 1989). Assuming the same number of 1400 pairs per tree, this would correspond to a release of 0.01 mg/h. Further studies from other bark beetle species and host trees are needed to provide information on ecologically relevant concentrations that are perceived by the beetles under natural conditions. In that regard, it is not yet clear how the concentrations measured in the gut relate to actual verbenone release rates. For *I. typographus*, it has recently been found that the aggregation pheromone component *cis*-verbenol can be stored as verbenyl oleate in the fat body (Ramakrishnan et al. 2022). This finding suggests that there might be an active regulation of pheromone emission by a controlled



release of these stored compounds. Future studies should link the amounts of pheromone components in the gut to actual release rates and extrapolate these values to the landscape scale. Finally, these findings should then be related to the bark beetles' perception by their olfactory system and resulting behaviour (Q2).

## Phenology of verbenone emission

If verbenone was a 'perfect' anti-aggregation pheromone, it should be expected not to occur at the early stages of an infestation by gregarious species (Fig. 2, solid blue line), because at this point mates are still needed to collectively overcome tree defence (Raffa and Berryman 1983). The



**Fig. 2** Schematic overview on the temporal verbenone release patterns from monogamous and polygynous bark beetles, (i) trajectory as expected for an ideal anti-aggregation pheromone to avoid overpopulation ('active' pathway, blue solid line), (ii) trajectory as expected if verbenone synthesis is a passive, unavoidable consequence of feeding activity ('passive' pathway, green solid line) or (iii) trajectories reported in the literature obtained from gut extracts (thin solid lines), phloem extracts (short-dashed lines) and atmospheric concentrations (long-dashed lines). Note that the developmental stages on the x-axis are evenly spaced for the sake of simplicity. In reality, these rela-

tive time spans would be species-dependent and strongly vary with ambient temperature. Amplitudes are relative within a study and not standardised among studies. All species presented here are gregarious except *D. rhizophagus*<sup>6</sup>. References: <sup>1</sup>Cale et al. (2015), <sup>2</sup>Pureswaran and Sullivan (2012), <sup>3</sup>Birgersson and Bergström (1989), <sup>4</sup>Shi and Sun (2010), <sup>5</sup>Xie and Lv (2012), <sup>6</sup>Cano-Ramírez et al. (2012), <sup>7</sup>Liu et al. (2019), <sup>8</sup>Zhang et al. (2009), <sup>9</sup>Birgersson et al. (1984), <sup>10</sup>Ramakrishnan et al. (2022), <sup>11</sup>Zhang et al. (2000), <sup>12</sup>Byers et al. (1984), <sup>13</sup>Xie and Lv (2013), <sup>14</sup>Leufvén and Birgersson (1987), <sup>15</sup>Taft et al. (2015)

amount of verbenone is then expected to increase immediately upon mating and further with an ongoing infestation in accordance with its putative function as an indicator of high colonisation densities and in order to shift an attack to a neighbouring tree (Renwick and Vité 1969; Rudinsky et al. 1974; Vité and Francke 1985; Byers 2004). But even in solitary bark beetle species, as well as ambrosia beetles, an attraction inhibitor like verbenone should not be released before mating was successful. In accordance, verbenone concentrations released from unmated females of the gregarious species *D. frontalis* were low and not significantly different from galleries with unsuccessful attacks, while the amount doubled once males joined the gallery (Pureswaran and Sullivan 2012). Also in the guts of freshly mated *D. valens* verbenone occurred in a similar high amount as *cis*- and *trans*-verbenol (Zhang and Sun 2006).

Several other studies, however, contradict this expected pattern by either showing that (i) verbenone amounts decline after construction of the nuptial chamber and/or mating, and that (ii) significant amounts of verbenone can already occur before mating (Fig. 2, thin solid lines). For instance, the amount of verbenone was reported to decrease after mating and during oviposition in both sexes of *D. rhizophagus* (Cano-Ramírez et al. 2012), *I. nitidus* (Zhang et al. 2009), *I. typographus* (Birgersson et al. 1984) and female *D. armandi* (Xie and Lv 2012).

Furthermore, verbenone has often been found at the early stages of the life cycle. For example, after maturation feeding but before emergence as in *I. typographus* (Ramakrishnan et al. 2022) or right after emergence as in *D. frontalis* (Grosman et al. 1997), *D. brevicomis* (Byers 1983a; Byers et al. 1984), *D. ponderosae*, and *I. pini* (Pureswaran et al. 2000). Some studies show that verbenone is also present some time after feeding has occurred, e.g. when landing on a tree (Byers et al. 1984) and during construction of the nuptial chamber (Zhang et al. 2007b, 2009; Xie and Lv 2013; Taft et al. 2015), which might be explained by feeding residues in the guts. However, callow beetles (during maturation feeding) of *D. brevicomis* of either sex did not contain verbenone in detectable amounts at this stage (Byers 1983b), but after dispersal when landing on a host pine tree (Byers et al. 1984) so that maturation feeding must have occurred in between or the production of verbenone from ingested host precursors occurred with some delay.

Instead of a steady increase in verbenone concentration over time, as observed in *D. ponderosae* during the first five days of colonisation (Cale et al. 2015), *T. piniperda* (Byers et al. 1989), *T. yunnanensis*, and *T. minor* (Wu et al. 2019), several studies suggest an early initial increase in verbenone, followed by a decline and then a steady increase in at least one sex (Birgersson and Bergström 1989; Taft et al. 2015; Liu et al. 2019; Ramakrishnan et al. 2022). These two resulting peaks might correlate with the production of ‘primary’

and ‘secondary resin’ as tree defence (Leufvén and Birgersson 1987). Moreover, the later increase may correspond to what would be expected in decaying host tissue even without bark beetles and/or their associated microorganisms in the form of autoxidation (Flechtmann et al. 1999; Dvořáková et al. 2007).

The fact that verbenone can often be found in bark beetles before mating challenges its role as a ‘perfect’ anti-aggregation pheromone. Instead, the occurrence of verbenone rather seems to be a passive consequence during phases of intense feeding activity instead of a controlled release of an ideal anti-aggregation pheromone (Fig. 2, solid green line; Pureswaran and Borden 2003; Shi and Sun 2010; Cano-Ramírez et al. 2012). This is in line with the strong linkage of verbenone to the host tissue quality and availability of the precursor compounds  $\alpha$ -pinene and verbenol (Shi and Sun 2010; Cano-Ramírez et al. 2012; Cale et al. 2015). Also from an energetic perspective, it might be favourable for the beetles to rely on host compounds as cues instead of *de novo* synthesis (Byers 1983a, 1989). According to the ‘sender-precursor hypothesis’, what we observe could be the transition phase of a semiochemical—with an original ecological meaning (‘elevated bark beetle activity’)—to an actual pheromone. This hypothesis postulates that the presence of a compound associated with a specific state (here: the intense feeding of conspecifics) causes the evolution of the receiver’s sensory capacities necessary to detect that cue and the employment as a pheromone (Stöckl and Steiger 2017). Assuming an ongoing evolution regarding the employment of verbenone, it is therefore possible that some beetle species can actively regulate biosynthesis (i.e. produce a signal), while others cannot (i.e. rely on a cue).

The finding that verbenone is synthesised to a large extent by associated microorganisms might further explain why verbenone is a semiochemical used by many scolytine species. At least some of the verbenone-synthesising yeasts, including *Candida nitratophila*, *Hansenula capsulate* and *Pichia pinus* were detected in the two geographically separated species *D. ponderosae* and *I. typographus* (Hunt and Borden 1990). In case microbial species are commonly shared among phylogenetically distant scolytine species, the ability of verbenone biosynthesis could be gained relatively quickly by incorporating the respective microbial species by means of horizontal transfer. The detection capability and response to verbenone could then be interpreted as an adaptation allowing to detect and respond to a ‘universal’ bark beetle microbiome.

Studies on the temporal occurrence of verbenone based only on extracts from adult beetles also may thus underestimate the amounts that occur in proximity to an infestation, particularly during advanced stages of an infestation, because they ignore verbenone originating from other sources outside the beetles, e.g. the gallery walls (Leufvén

and Birgersson 1987). This issue should not arise from headspace samplings that inherently include volatiles emitted from any source. It must therefore be assumed that volatile samplings, contrary to extracts, more accurately reflect the verbenone concentrations that are relevant for newly arriving mates. Verbenone amounts in headspace samples indeed increased for *D. ponderosae* from host acceptance to oviposition (Cale et al. 2015) and in *I. typographus* after mating (Birgersson and Bergström 1989), but not in *D. frontalis* where the concentration increased before mating and declined again afterwards (Pureswaran and Sullivan 2012). In *I. typographus*, also the verbenone concentration inside the phloem showed a strong increase with the onset of oviposition (Leufvén and Birgersson 1987). Byers et al. (1989) observed a continuous increase in verbenone released from artificially inoculated windthrown pines with *T. piniperda* during the first five days and commented that this does not match with observations from gut extracts. It therefore seems that the expected increase in verbenone with later stages of bark beetle colonisation to indicate unsuitable habitat rather originates from sources outside the beetles, suggesting once more the importance of associated microorganisms.

So far, only one study investigated verbenone concentrations associated with larval frass. In the laboratory, phloem extracts had significantly higher proportions of verbenone when *D. valens* larvae were also present and larval frass extracts from *Dendroctonus micans* and *D. valens* collected in the field both contained verbenone (Grégoire et al. 1991). Thus, also larval activity may contribute to the release of verbenone and is another potential source not captured by gut analysis of adult beetles, possibly leading to an underestimation of relevant verbenone amounts. However, most studies ended before substantial larval activity could occur (i.e. they did not observe later than early oviposition) so that this bias is of low relevance for the literature comparison that is presented here. If the release of verbenone from larval frass occurs in the host tree, this may serve to prevent colonisation of the same tree by newly arriving beetles and also trigger the dispersal of the emerging beetles away from the maternal tree to prevent inbreeding.

According to the strict definition, pheromones are chemical signals that act within a species (Wyatt 2010). The findings here suggest the importance of associated microorganisms in the verbenone synthesis and sources outside the beetles. This obviously contradicts the concept of a pheromone *sensu stricto* requiring the sender and receiver to be from the same species. According to the classification of semiochemicals, verbenone must therefore rather be classified as an allelochemical if produced by microorganisms and perceived by bark beetles (Nordlund and Lewis 1976). Six (2013) advocates the concept of bark beetles as a ‘holobiont’, i.e. the entity of a bark beetle and its associated microorganisms, which may be more useful in this context

than the taxonomic unit ‘species’ in case a more accurate functional understanding of the ecology of bark beetles is needed. This concept also seems useful to be applied in regard to verbenone, because even if the origin of this compound lies outside the beetles’ bodies, the relevant biological agents are clearly associated with bark beetle colonisation.

Interestingly, verbenone is not the only example for compound pairs of which the terpene alcohol is an attractant and the corresponding ketone a repellent. For example, seudenol (3-methyl-2-cyclohexen-1-ol) is part of the aggregation pheromone of *D. pseudotsugae* and seudenone (3-methyl-2-cyclohexen-1-one) is inhibitory (Brand et al. 1976; Foote et al. 2020). Quercivorol ((1S,4R)-4-isopropyl-1-methyl-2-cyclohexen-1-ol) is an attractant for the species complex *Euwallacea fornicatus* and piperitone (6-isopropyl-3-methyl-1-cyclohex-2-enone), the ketone of a constitutional isomer of quercivorol, and significantly lowered the attractiveness in a baited trap experiment (Dodge et al. 2017; Byers et al. 2018, 2020). This phenomenon might be explained by a parsimonious use of chemical compounds on one hand, and a well-preserved chain of behaviours within the scolytine beetles that includes attraction at the early stages of colonisation and anti-aggregation at later stages.

## Verbenone reception and behavioural response

### Q2.1: Olfaction and neurophysiology—How is verbenone detected?

#### Antennal and neuronal response

The antennal response to verbenone, as measured via electroantennograms (EAG) or gas chromatographic–electroantennographic detection (GC-EAD), is well established across several genera (Whitehead 1986; Pureswaran et al. 2000; Schlyter et al. 2000; Zhang et al. 2006; Shepherd et al. 2007; Zhang et al. 2007a, b; Andersson 2012; López et al. 2013; Ranger et al. 2014; Kandasamy et al. 2023). On the neurological level, both sexes tend to respond in a similar way (Ascoli-Christensen et al. 1993; Zhang et al. 2006; Cano-Ramírez et al. 2012; Shepherd and Sullivan 2013; Zhao et al. 2017), even though *D. armandi* showed a sex-specific response to different verbenone amounts (Zhao et al. 2017).

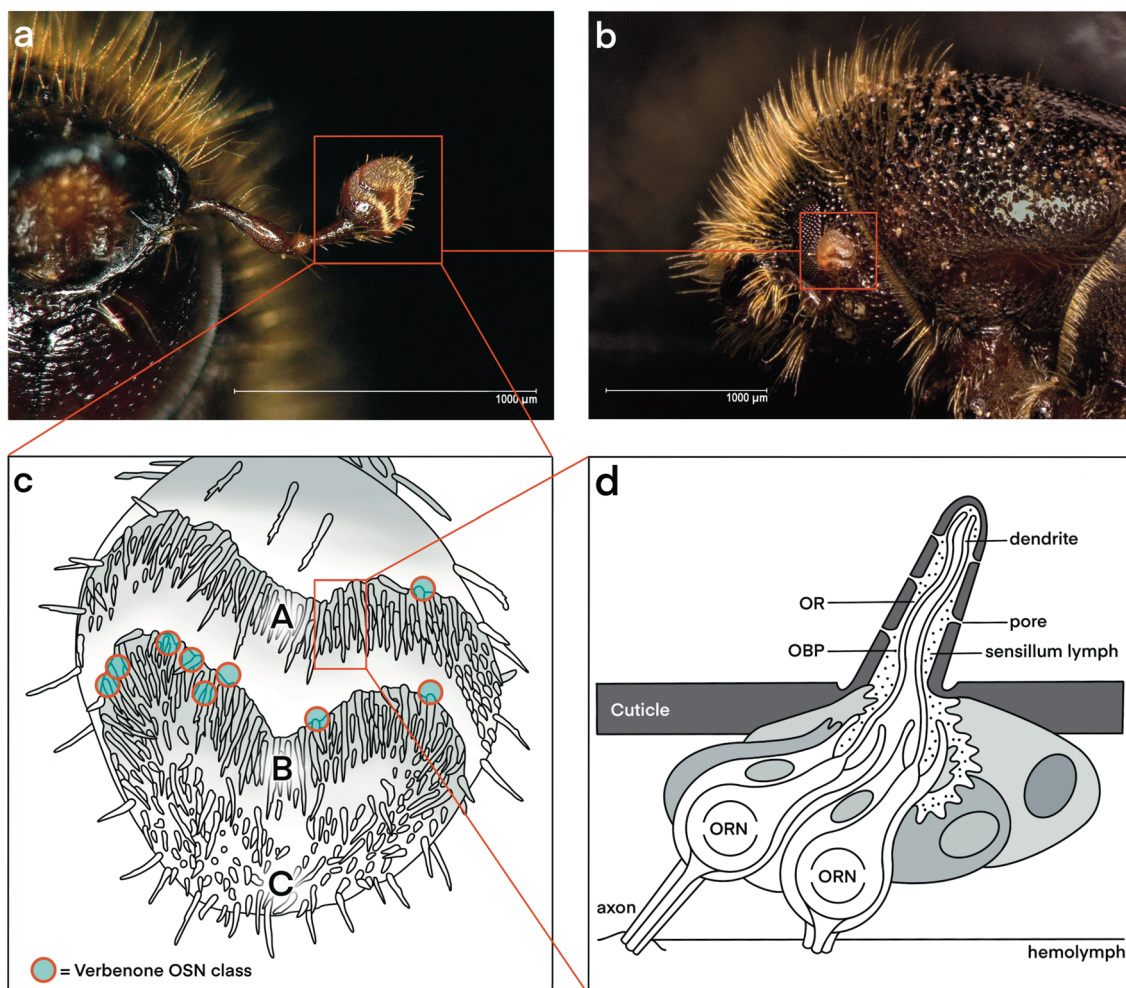
Unfortunately, the comparison of actual response threshold concentrations among species is nearly impossible for several reasons. Enantiomers, if not differentiated in a study—as is often the case—can function as different compounds and may possess completely different antennal detection thresholds (Dickens 1979). Comparison is further impeded since studies differ in test concentrations, the time span in between two stimuli, and the order in which different

dosages are presented. Different solvents are used, which can affect the electrophysiological responses that are presented as absolute or often as normalised output relative to controls of different quality. Thus, it is unclear what the actual concentration of a compound is that reaches the antenna in a given study, and how this concentration compares to relevant concentrations in nature, making comparisons among studies difficult (Andersson et al. 2012).

### Signal processing and interaction with other stimuli

Even though it is well established that verbenone elicits a neuronal response in bark beetle antenna, it is not fully

clear how the signal is further processed and how it interacts with other incoming stimuli, e.g. attractive host cues or aggregation pheromones. Odour detection by olfactory receptor neurons (ORNs) in bark beetles is summarised in Andersson (2012). In brief, the responsible organs are the antennae (Fig. 3) and, to a lesser extent, the maxillary palps. They contain olfactory sensilla, which are hair-like structures housing the ORNs. These neurons express the odorant receptors (ORs) that bind the odour molecules to trigger neuronal responses. It is further thought that odorant-binding proteins (OBP) (Dickens 1997), also called pheromone binding proteins when a pheromone is the target compound (Breer 1997), may play an important



**Fig. 3** Odour detection at the example of *Ips typographus*. The bark beetle antenna **a**, **b** contains olfactory sensilla which are organised **c** in two distinct undulating bands **A**, **B** and one distal area **C**. The sensilla containing the olfactory receptor neurons (ORN) that respond to verbenone have been found primarily in **B**, to a lower extent in **A** and they appear to be completely missing in **C**. One sensillum generally contains two ORNs which house the odorant receptor (OR) membrane proteins in the dendrites of these neurons **d**. An OR that pri-

marily responds to verbenone has so far not been identified and it is not yet clear whether odorant binding proteins (OBPs) play a role in the detection of verbenone. The inhibitory effect of verbenone on the attraction to the aggregation pheromone is most likely caused by integration of the incoming stimuli in the central nervous system. (Figure modified from Jacquin-Joly and Merlin (2004), Andersson et al. (2009) and Kandasamy et al. (2023), figure credit to Fenris Måling)

role for a compound to solubilise and get access to the OR. Recently, MaltOBP1 has been identified in the long-horn beetle *Monochamus alternatus* with a high binding affinity to (+)- $\alpha$ -pinene and (–)-verbenone (Zhang et al. 2020). Interestingly, its expression was not limited to the antennae, but also occurred in the legs, wings and to some degree also in other parts of the body. To our knowledge, it is still an open question whether OBPs that bind verbenone exist also in bark beetles and whether the detection of verbenone is limited to the antenna.

To act as an inhibitor (see Q2.2), verbenone must somehow interfere with an attracting stimulus. This can either occur inside the olfactory organs i) as ‘peripheral inhibition’, e.g. by direct interference with ORs for attractive stimuli or by the interactions between ORNs housed inside the same sensilla (Andersson et al. 2010; Su et al. 2012), or ii) in the central nervous system by central integration of attractive and aversive signals (Visser and de Jong 1988; Byers 1989). In *I. typographus*, an ORN class responding specifically to verbenone was originally reported by Tømmerås (1985) and later by Andersson et al. (2009). However, in more recent single sensillum recordings by Kandasamy et al. (2023) including additional monoterpene ketones, the same ORN class also showed similar responses to  $\alpha$ - and  $\beta$ -isophorone (the latter reported in low amounts in the gut of mated females; Birgersson et al. 1984), suggesting that verbenone detection is less specific than previously thought. The verbenone-responsive neurons are not co-localised in the same sensilla as neurons detecting the aggregation pheromone components *cis*-verbenol or 2-methyl-3-methyl-2-ol, respectively; several of the *cis*-verbenol neurons are instead co-localised with ORNs detecting the inhibitory spruce defence compound 1,8-cineole (Andersson et al. 2010). This in combination with the fact that verbenone does not inhibit the ORN classes that detect aggregation pheromone components suggests that the verbenone stimulus is most likely integrated centrally in the nervous system and thus activates neuronal circuits responsible for an aversive behavioural response. In addition to the verbenone-responsive ORN class thoroughly characterised in *I. typographus*, neurons primarily responding to this compound have been reported also in other species such as *I. pini*, *D. pseudotsugae*, and *D. frontalis* (summarised in Andersson 2012). Thus far, an OR that is responsible for the recorded ORN responses to verbenone remains to be identified. Certain compounds (i.e.  $C_6$  green leaf volatiles and 2-phenylethanol) that are ecologically relevant for several species within the Curculionidae were recently shown to activate evolutionarily conserved ORs with highly similar response specificities in different species (Roberts et al. 2022). The widespread use of verbenone among scolytine beetles begs the question of whether this compound also is detected by an OR that has been conserved across this beetle subfamily.

Understanding the neurological signal transduction in response to an olfactory stimulus is only the first step. But how does the change in neuro-activity affect the beetle’s physiology? This question has been even less addressed so far and remains speculative. Dickens and Payne (1978) found a reduced muscle potential in the antenna of freshly emerged *D. frontalis* as an immediate response to *trans*-verbenol and verbenone. However, it is unlikely that a single change in muscle potential will affect the complex host search behaviour. Byers et al. (2004) observed flying *P. bidentatus* actively avoiding baited traps with additional non-host tissue by changing the direction of flight when approaching the traps by 0.5–1 m. An active flight response away from a non-attractive cue also seems more logical than an inhibition of the flight muscles, which would not allow the beetles to actually avoid non-suitable hosts. It therefore seems that the behavioural response to verbenone involves a complex—and largely unknown—interplay of a multitude of distinct factors, probably also including the physiological and nutritional state of an individual.

## Q2.2: What is the behavioural response of bark beetles to verbenone and which factors influence this response?

### Behavioural response to verbenone

For at least 45 scolytine species (45/53 species that were studied) from 18 different genera, a behavioural response to verbenone was described (Table 2). Most reports refer to coniferophagous bark beetles, but also some angiosperm-feeding species (e.g. Kohnle et al. 1992; Audley et al. 2020) and ambrosia beetles (e.g. Ranger et al. 2014; Byers et al. 2020, 2021; Martini et al. 2020) respond to verbenone.

Most studies tested for, and also proved, ‘inhibition’ by verbenone (Table 2, Fig. 4), which is defined here as the reduced or completely suppressed attraction response to an otherwise attractive source (Table 3). Experimental release rates of verbenone point sources range from only ~1 mg/d (e.g. Erbilgin et al. 2008; Burbano et al. 2012; Byers et al. 2018, 2020) up to ~100 mg/d (Bertram and Paine 1994a; Hayes et al. 1994; Gillette et al. 2014), but most of the times intermediate release rates around 20–50 mg/d were used (e.g. Borden et al. 1992; Fettig et al. 2013; Strom et al. 2013; Agnello et al. 2017; Audley et al. 2020). Inhibition was most prominently studied in the economically important species *D. ponderosae*, *D. frontalis*, *D. brevicomis* and *I. typographus* (Table 2). While most work confirms the inhibitory effect of verbenone, some studies did not find an inhibitory effect (e.g. Niemeyer et al. 1995; Jakuš et al. 2003; Shepherd and Sullivan 2013). From 53 species for which we found relevant studies, 38 (~72%) were inhibited by verbenone (Table 2). The proportion was slightly larger

Table 2 Behavioural response of bark and ambrosia beetles to verbenone

| Species                         | Mating system and host use strategy* | Group           | Enantiomer           | Effect of verbenone against attractant | Effect of verbenone in blend             | Responding sex | Affected behaviour              |                          | References   |
|---------------------------------|--------------------------------------|-----------------|----------------------|--|--|----------------|---------------------------------|--------------------------|--|
|                                 |                                      |                 |                      |  |  |                | Walking                         | Flying                   |  |
| <i>Anisandrus mitchae</i>       | Ib, S                                | Ambrosia beetle | (-)                  | Strongly inhibitory                    | -  | Females        | -                               | Yes                      | Tobin and Ginzel (2022)  |
| <i>Anisandrus sayi</i>          | Ib, S                                | Ambrosia beetle | -                    | Inhibitory                             | -  | Females        | -                               | Yes                      | Ranger et al. (2013)   |
| <i>Conophthorus conicolens</i>  | Mg                                   | Cone breeder    | 85%(-)               | Weakly inhibitory                      | Synergistic inhibitor                    | Males          | -                               | Yes                      | Rappaport et al. (2000)  |
| <i>Conophthorus coniperda</i>   | Mg                                   | Cone breeder    | 85, 87%(-)           | None-weakly inhibitory                 | Weak synergistic inhibitor               | Males          | -                               | (Yes)                    | de Groot and DeBarr (2000), Rappaport et al. (2000)  |
| <i>Conophthorus ponderosae</i>  | Mg                                   | Cone breeder    | 85%(-)               | None                                   | Weak synergistic inhibitor or attractant | Males          | -                               | (Yes)                    | Rappaport et al. (2000)  |
| <i>Conophthorus resinosa</i>    | Mg                                   | Cone breeder    | 85%(-)               | None                                   | None                                     | Males          | -                               | No                       | Rappaport et al. (2000)  |
| <i>Conophthorus teocotum</i>    | Mg                                   | Cone breeder    | 85%(-)               | None                                   | None                                     | Males          | -                               | No                       | Rappaport et al. (2000)  |
| <i>Corthylus columbianus</i>    | Mg, P                                | Ambrosia beetle | (-)                  | -                                      | -  | Females        | -                               | Not repellent/attractive | VanDerLaan and Ginzel (2013)   |
| <i>Dendroctonus armandi</i>     | Mg, P                                | Bark beetle     | 82%(-)               | Moderately inhibitory                  | -  | Both           | Yes, sex-specific dose response | Yes                      | Zhao et al. (2017)   |
| <i>Dendroctonus brevicornis</i> | Mg, P                                | Bark beetle     | 77-97%(-), 60-69%(+) | Weakly-strongly inhibitory             | Synergistic inhibitor                    | Both           | -                               | Yes                      | Tilden and Bedard 1988, Paine and Hanlon (1991), Dickens et al. (1992), Bertram and Paine (1994a, b), Hayes and Strom (1994), Strom et al. (2001), Fettig et al. (2005, 2009a, 2012a, b), Erbilgin et al. (2007), Erbilgin et al. (2008) |

Table 2 (continued)

| Species                       | Mating system and host use strategy* | Group       | Enantiomer                   | Effect of verbenone against attractant | Effect of verbenone in blend | Responding sex                    | Affected behaviour |        | References   |
|-------------------------------|--------------------------------------|-------------|------------------------------|--|------------------------------|-----------------------------------|--------------------|--------|--|
|                               |                                      |             |                              |  |                              |                                   | Walking            | Flying |  |
| <i>Dendroctonus frontalis</i> | Mg, P                                | Bark beetle | 66–83%-(−), 69%-(+), racemic | Strongly inhibitory                    | Synergistic inhibitor        | Both, male with stronger response | (Yes)              | Yes    | Renwick and Vité (1969), Richerson and Payne (1979), Payne and Billings (1989), Payne et al. (1992), Salom et al. (1992), Salom et al. (1995), Hayes et al. (1994), Salom et al. (1997), Clarke et al. (1999), Strom et al. (1999), Sullivan et al. (2007), Shepherd and Sullivan (2013) |
| <i>Dendroctonus jeffreyi</i>  | Mg, P                                | Bark beetle | (−)                          | Strongly inhibitory                    | Synergistic inhibitor        | Both                              | –                  | Yes    | Strom et al. (2013)  |
| <i>Dendroctonus mexicanus</i> | Mg, S                                | Bark beetle | (−), (+)                     | (Inhibitory)                           | –                            | Not differentiated                | –                  | (Yes)  | Díaz-Núñez et al. (2006)   |

Table 2 (continued)

| Species                         | Mating system and host use strategy* | Group        | Enantiomer              | Effect of verbenone against attractant | Effect of verbenone in blend | Responding sex | Affected behaviour                                  |        | References   |
|---------------------------------|--------------------------------------|--------------|-------------------------|--|------------------------------|----------------|---|--------|--|
|                                 |                                      |              |                         |  |                              |                | Walking   | Flying |  |
| <i>Dendroctonus ponderosae</i>  | Mg, P                                | Bark beetle  | ≥ 50%-(−),<br>70.4%-(+) | Strongly inhibitory                    | Synergistic inhibitor        | Both           | Yes   | Yes    | Ryker and Yandell (1983), Lindgren et al. (1989), Schmitz and McGregor (1990), Amman et al. (1991), Gibson et al. (1991), Safranyik et al. (1992), Shea et al. (1992), Shore et al. (1992), Lindgren and Borden (1993), Hayes and Strom (1994), Miller et al. (1995), Borden et al. (1998, 2003, 2006), Huber and Borden (2001), Lindgren and Miller (2002b), Progar (2003, 2005), Benitz et al. (2005), Gillette et al. (2006), Gillette et al. (2009), Gillette et al. (2012a, b, 2014), Perkins et al. (2011, 2015), Fettig et al. (2012a, 2015, 2020), Progar et al. (2013, 2021), Fettig and Munson (2020) and Cano-Ramirez et al. (2012) |
| <i>Dendroctonus rhizophagus</i> | Mg, P                                | Bark beetles | 92%-(−)                 | Inhibitory                             | Synergistic inhibitor        | Males          | –   | Yes    | Rappaport et al. (2001), Sun et al. (2003), Gillette et al. (2006), Zhang and Sun (2006), Zhang et al. (2006), Fettig et al. (2007, 2008), Strom et al. (2013),  |
| <i>Dendroctonus valens</i>      | Mg, Parasite                         | Bark beetle  | 80–98%-(−)              | Strongly inhibitory                    | Synergistic inhibitor        | Both           | Yes (tendency of attraction for low concentrations) | Yes    | Yes (only blend tested)  |



Table 2 (continued)

| Species                                  | Mating system and host use strategy* | Group           | Enantiomer                | Effect of verbenone against attractant | Effect of verbenone in blend | Responding sex                     | Affected behaviour |               | References  |
|--|--------------------------------------|-----------------|---------------------------|--|------------------------------|------------------------------------|--------------------|---------------|---|
|  |                                      |                 |                           |  |                              |                                    | Walking            | Flying        |   |
| <i>Eiwallacea fornicatus</i>             | Ib, P                                | Ambrosia beetle | 74%(-), 75–89%(+)         | Strongly inhibitory                    | Synergistic inhibitor        | Females                            | –                  | Yes           | Dodge et al. (2017), Byers et al. (2018, 2020, 2021)                          |
| <i>Eiwallacea validus</i>                | Ib, P                                | Ambrosia beetle | 78%(-)                    | Weakly–strongly inhibitory             | –                            | Females                            | –                  | Yes           | Ranger et al. (2013), Ranger et al. (2014))                                   |
| <i>Gnathotrichus materiarius</i>         | Ib, S                                | Ambrosia beetle | 78%(-)                    | None–strongly inhibitory               | –                            | Not differentiated                 | –                  | Yes           | Dodds and Miller (2010)   |
| <i>Hylurgops longicollis</i>             | Mg, S                                | Bark beetle     | 83%(-)                    | None                                   | –                            | Not differentiated                 | –                  | No            | Lindgren and Miller (2002b)   |
| <i>Hylurgops palliatus</i>               | Mg, S                                | Bark beetle     | 83–99.2%(-)               | None(attractant)                       | (No)                         | Both                               | –                  | (Yes)         | Byers (1992a), Schlyter et al. (2000), Romón et al. (2007)                    |
| <i>Hylurgops porosus</i>                 | Mg, S                                | Bark beetle     | 83%(-)                    | None                                   | –                            | Not differentiated                 | –                  | No            | Lindgren and Miller (2002b)   |
| <i>Hylurgops ligniperda</i>              | Mg, S                                | Bark beetle     | 83%(-)                    | None–inhibitory                        | Synergistic attractant       | Not differentiated                 | –                  | No            | Etxebeeste and Pajares (2011), Etxebeeste et al. (2013)                       |
| <i>Hypothenemus dissimilis</i>           | Ib, S                                | Ambrosia beetle | n.r                       | Inhibitory                             | –                            | Not differentiated                 | –                  | Yes           | Ranger et al. (2013)  |
| <i>Hypothenemus hampei</i>               | Ib                                   | Seed breeder    | n.r                       | Repellent                              | –                            | Females                            | Yes                | –             | Jaramillo et al. (2013)   |
| <i>Ips duplicatus</i> (Asian population) | Pg, P                                | Bark beetle     | n.r                       | Moderately–strongly inhibitory         | Synergistic inhibitor        | Both, males with stronger response | –                  | Yes           | Zhang et al. (2001)   |
| <i>Ips grandicollis</i>                  | Pg, P                                | Bark beetle     | 78%(-)                    | None–synergistic attractant            | –                            | Not differentiated                 | –                  | Yes           | Dodds and Miller (2010)   |
| <i>Ips latidens</i>                      | Pg, P                                | Bark beetle     | 83%(-)                    | Moderately–strongly inhibitory         | –                            | Both                               | –                  | Yes           | Miller et al. (1995), Lindgren and Miller (2002b)                             |
| <i>Ips paraconfusus</i>                  | Pg, P                                | Bark beetle     | 84–99.5%(-), 68–98.3%(-+) | Moderately–strongly inhibitory         | Synergistic inhibitor        | Both                               | Yes                | Yes           | Byers and Wood (1980, 1981), Paine and Hanlon (1991), McPherson et al. (1997) |
| <i>Ips perturbatus</i>                   | Pg, P                                | Bark beetle     | 77–84%(-)                 | None                                   | Synergistic inhibitor        | Not differentiated                 | –                  | Yes, in blend | Graves et al. (2008), Fetting et al. (2013)                                   |

Table 2 (continued)

| Species  | Mating system and host use strategy* | Group       | Enantiomer              | Effect of verbenone against attractant | Effect of verbenone in blend | Responding sex                       | Affected behaviour |        | References   |
|--|--------------------------------------|-------------|-------------------------|--|------------------------------|--------------------------------------|--------------------|--------|--|
|  |                                      |             |                         |  |                              |                                      | Walking            | Flying |  |
| <i>Ips pini</i>  | Pg, P                                | Bark beetle | 50–83%(-)               | Moderately–strongly inhibitory         | Synergistic inhibitor        | Both                                 | –                  | Yes    | Borden et al. (1992), Devlin and Borden (1994), Miller et al. (1995), Lindgren and Miller (2002b), Sallé and Raffa (2007), Dodds and Miller (2010), Gaylord (2020, 2023) |
| <i>Ips sexdentatus</i>                                     | Pg, P                                | Bark beetle | 83%(-)                  | Moderately–strongly inhibitory         | Synergistic inhibitor        | Not differentiated                   | –                  | Yes    | Kohnle et al. (1992), Etxebeste and Pajares et al. (2013)  |
| <i>Ips subelongatus</i>                                    | Pg, P                                | Bark beetle | (-)                     | (Strongly inhibitory)                  | –                            | Both                                 | –                  | (Yes)  | Zhang et al. (2007a)   |
| <i>Ips typographus</i>                                     | Pg, P                                | Bark beetle | 75.2%(-), (-), 76.5%(+) | Weakly–strongly inhibitory             | Synergistic inhibitor        | Both, males with stronger response   | Yes                | Yes    | Bakke (1987), Schlyter et al. (1989), Zhang et al. (1999), Jakuš et al. (2003), Faccoli et al. (2005), Schiebe et al. (2011), Unelius et al. (2014)                      |
| <i>Leperisinus varius</i> (now: <i>Hylesinus fraxini</i> ) | Mg, P                                | Bark beetle | (-)                     | Moderately–strongly inhibitory         | –                            | Not differentiated                   | –                  | Yes    | Kohnle et al. (1992)   |
| <i>Orthotomicus erosus</i>                                 | Pg, P                                | Bark beetle | 83%(-)                  | Strongly attractive                    | –                            | Not differentiated                   | –                  | –      | Etxebeste et al. (2013)  |
| <i>Pityogenes chalcographus</i>                            | Pg, P                                | Bark beetle | 99.2%(-)                | (Moderately inhibitory)                | –                            | Both, females with stronger response | –                  | (Yes)  | Byers (1993)   |
| <i>Pityophthorus juglandis</i>                             | Pg, P                                | Bark beetle | (-), (+)                | Strongly inhibitory                    | Synergistic inhibitor        | Both, males with stronger response   | –                  | Yes    | Audley et al. (2020)   |
| <i>Pityophthorus pubescens</i>                             | Mg, S                                | Bark beetle | (-)                     | Strongly inhibitory                    | –                            | Both                                 | –                  | Yes    | López et al. (2013)  |
| <i>Tomiscus brevipilosus</i>                               | Mg, S                                | Bark beetle | n.r                     | Weakly attractive                      | –                            | Both                                 | Yes                | –      | Liu et al. (2019)  |

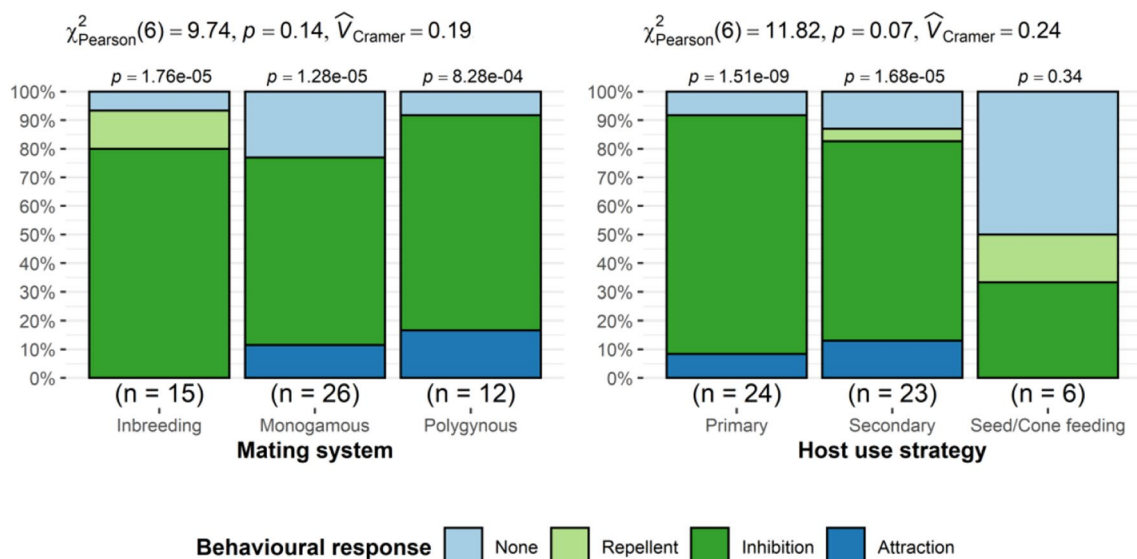
Table 2 (continued)

| Species                          | Mating system and host use strategy* | Group           | Enantiomer | Effect of verbenone against attractant | Effect of verbenone in blend | Responding sex     | Affected behaviour |        | References  |
|----------------------------------|--------------------------------------|-----------------|------------|--|------------------------------|--------------------|--------------------|--------|---|
|                                  |                                      |                 |            |  |                              |                    | Walking            | Flying |   |
| <i>Tomticus minor</i>            | Mg, S                                | Bark beetle     | n.r        | Strongly inhibitory                    | Synergistic inhibitor        | Both               | Yes                | Yes    | Schlyter et al. (2000), Wu et al. (2019)  |
| <i>Tomticus piniperda</i>        | Mg, S                                | Bark beetles    | 83–84%(-)  | Weakly–strongly inhibitory             | Synergistic inhibitor        | Not differentiated | Yes                | Yes    | Byers et al. (1989), Kohnle et al. (1992), McCullough et al. (1998), Schlyter et al. (2000), Poland et al. (2004) |
| <i>Tomticus yunnanensis</i>      | Mg, S                                | Bark beetle     | n.r        | Strongly inhibitory                    | –                            | Both               | Yes                | Yes    | Wu et al. (2019)  |
| <i>Trypodendron domesticum</i>   | Mg, S                                | Ambrosia beetle | 99.2%(-)   | Moderately inhibitory                  | –                            | Both               | –                  | Yes    | Byers (1992a)   |
| <i>Trypodendron lineatum</i>     | Mg, S                                | Ambrosia beetle | 83%(-)     | Neutral (– moderately attractive)      | –                            | Not differentiated | –                  | (Yes)  | Lindgren and Miller (2002a)   |
| <i>Xyleborinus saxesenii</i>     | Ib, S                                | Ambrosia beetle | 84%(-)     | Strongly inhibitory                    | –                            | Females            | –                  | Yes    | Burbano et al. (2012), Ranger et al. (2013)   |
| <i>Xyleborus atratus</i>         | Ib, S                                | Ambrosia beetle | 78%(-)     | (Weakly inhibitory)                    | –                            | Not differentiated | –                  | (Yes)  | Ranger et al. (2014)  |
| <i>Xyleborus bispinatus</i>      | Ib, S                                | Ambrosia beetle | n.r        | Weakly repellent                       | –                            | Females            | (Yes)              | –      | Rivera et al. (2020)  |
| <i>Xyleborus glabratus</i>       | Ib, P                                | Ambrosia beetle | n.r        | Weakly–strongly inhibitory             | Synergistic inhibitor        | Females            | –                  | (Yes)  | Hughes et al. (2017), Martini et al. (2020)   |
| <i>Xyleborus volvolus</i>        | Ib, S                                | Ambrosia beetle | n.r        | None                                   | –                            | Females            | No                 | –      | Rivera et al. (2020)  |
| <i>Xylosandrus compactus</i>     | Ib, S                                | Ambrosia beetle | 84%(-)     | Moderately inhibitory                  | –                            | Females            | –                  | Yes    | Burbano et al. (2012)   |
| <i>Xylosandrus crassiusculus</i> | Ib, S                                | Ambrosia beetle | 84%(-)     | Strongly inhibitory and repellent      | –                            | Females            | –                  | Yes    | Burbano et al. (2012), VanDerLaan and Ginzel (2013)   |
| <i>Xylosandrus germanus</i>      | Ib, S                                | Ambrosia beetle | (–)        | None–strongly inhibitory and repellent | –                            | Females            | –                  | Yes    | Dodds and Miller (2010), Ranger et al. (2013), VanDerLaan and Ginzel (2013), Agnello et al. (2017)                |

\*Mating system: Mg = monogamous, Pg = polygynous, Ib = inbreeding; host use strategy: P = primary, S = secondary, parasitic; statement in parenthesis when based on an extremely low sample size ( $\approx \leq 100$  individuals in total), if the study was not targeted at the mentioned species (e.g. species occurred as by-catch but the blend was not specific) or in case of other concerns regarding that piece of information

**Table 3** Definition of possible effects of semiochemicals considered for this review. Modified according to Dethier et al. (1960)

| Effect     | Compound   | Description of resulting beetle behaviour   |
|------------|------------|---|
| Attraction | Attractant | Active movement to the odour source (Dethier et al. 1960)   |
| Repellence | Repellent  | Active movement away from the odour source (i.e. no attraction involved) (Dethier et al. 1960)  |
| Inhibition | Inhibitor  | Attraction inhibition, i.e. the active movement to an attractive source is hampered in the presence of the inhibitor (modified according to, e.g. Byers et al. 1984; Renwick and Vité 1969; Vité and Baader 1990), ‘disruptant’ (e.g. Strom et al. 2013) or ‘anti-attractant’ may be used synonymously, while ‘antiaggregant’ (e.g. Strom et al. 2001) would only refer to gregarious species and should therefore be avoided in a more general context |



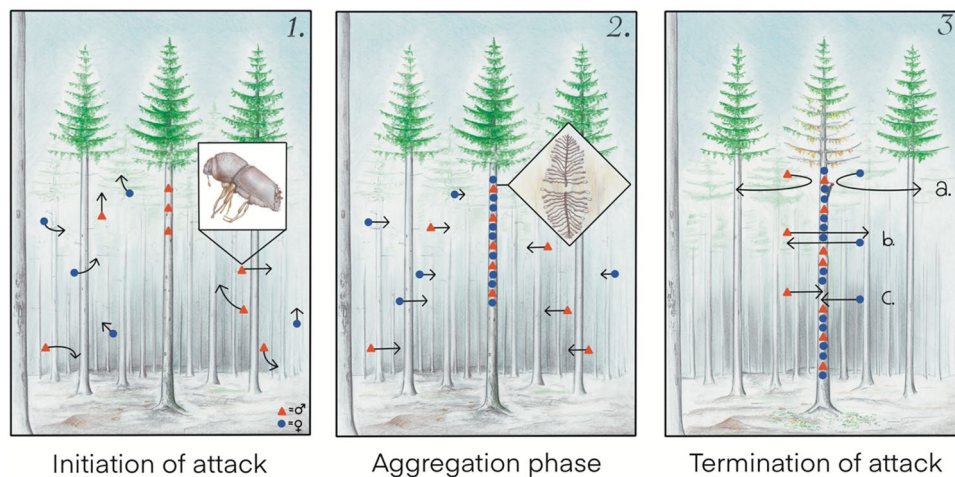
**Fig. 4** Interaction of the mating system (left) and host use strategy (right) of selected scolytine beetles (53 species for which relevant studies were available) with their behavioural response to verbenone as summarised from the literature (see Table 2). Qualitative classification of bark beetle species for all three parameters (response to verbenone, mating system, and host use strategy) was made accord-

ing to the information presented in Table 2; if multiple response types were reported for a species, the most common one was considered. Chi-square test was used for testing statistical significance among and within groups and Cramér’s V for estimation of the effect size (*R* package: ggstatsplot, Patil (2021))

in primary (20/24  $\approx$  83%) compared to secondary species (16/23  $\approx$  70%). Verbenone never completely inhibits the response to attractants, and only in some studies the attraction of baited traps was reduced to a level similar to that of blank controls (e.g. Ranger et al. 2013; Audley et al. 2020) or more than 90% (e.g. Tilden and Bedard 1988; Hayes and Strom 1994). In other cases, the reduction was around or below 50% (e.g. Strom et al. 1999; Zhang et al. 1999, 2006; Fettig et al. 2009b; Zhao et al. 2017). Accordingly, also attacks on host trees treated with verbenone are typically not completely suppressed (e.g. Lindgren et al. 1989; Bertram and Paine 1994a; Gillette et al. 2012a). The fact that some beetle individuals ‘ignore’ the verbenone signal (Fig. 5) is also supported by the finding from several *Dendroctonus* species that trees protected with pure verbenone or verbenone-containing blends often show a greater number of unsuccessful attacks: the number of attacking beetles is

reduced to a level so that tree defence cannot be overcome and comparatively more beetles—namely those ignoring verbenone—die in the resin (Shea et al. 1992; Lindgren and Borden 1993; Fettig et al. 2009a).

‘Attraction’ to pure verbenone or ‘repellence’ (see Table 3 for definitions) have been studied considerably less, requiring an approach that includes non-baited (passive) traps with verbenone as the only compound tested. Pure verbenone was neither attractive to several *Conophthorus* species in the field (de Groot and DeBarr 2000; Rappaport et al. 2000), nor to *I. paraconfusus* in a walking assay (McPheron et al. 1997). However, verbenone was shown to synergise attraction in two scenarios, i) for some secondary species and ii) for gregarious species at specific release rates or specific phases within the population dynamics. For example, verbenone only showed a weak effect in preventing infestation and brood development



**Fig. 5** Simplified host finding process at the example of the Eurasian spruce bark beetle (*Ips typographus*). **1.** In a first step, male pioneer beetles find suitable hosts by chance or guided by primary attraction. **2.** Once they establish, they produce aggregation pheromones to attract conspecifics of both sexes. **3.** Under the presence of verbenone (at later stages of the colonisation) further individuals are

either repelled **a** or their response to the aggregation pheromone is inhibited **b**, both resulting in a decrease in landing and attacks on the tree. Some individuals, however, ignore or do not manage to avoid the verbenone and continue landing on the tree **c**. (Modified according to Vité and Francke (1985), figure credits to Fenris Måling)

on pine stumps of the mostly secondary *T. piniperda* (McCullough et al. 1998, but see Schlyter et al. 1988), and *Hylurgops palliatus* (Byers 1992a). Livingston et al. (1983) found more *Hylurgops planirostris* in traps around pine bolts naturally infested with *D. adjunctus* when treated with verbenone, but only 19 beetles were caught altogether. Attraction of *H. palliatus* to synthetic pine kairomones was increased in one of four similar experiments when also verbenone was present and significantly different from non-baited control traps (Schlyter et al. 2000). Similarly, verbenone also increased the attractiveness of baited traps to *Trypodendron lineatum* (Lindgren and Miller 2002a). Lindgren and Miller (2002b) studied the response to verbenone of five bark beetle species with different levels of aggressiveness and found that aggressive species were inhibited, while trap catches of secondary species were not affected by verbenone. They concluded that verbenone acts as a signal for decaying wood for primary species that depend on fresh host material, while secondary species are indifferent to verbenone. A quantitative analysis of the collected literature supports this hypothesis by showing a slightly greater proportion of non-responding species and species attracted to verbenone among secondary species (6/23  $\approx$  26% of species tested) compared to primary species (4/24  $\approx$  17%, Fig. 4). A high proportion of species that are indifferent to verbenone was also found among six cone and seed feeding species (Fig. 4, but only for six species the response has been tested), suggesting that the host use behaviour (i.e. primary, secondary, cone/seed feeding) is to some extent related to the behavioural response to verbenone.

Furthermore, verbenone synergizes attraction to aggregation pheromones for primary species at specific release rates or specific phases within the population dynamics. The synergistic attraction was observed for *C. ponderosae* (Rappaport et al. 2000), but was not consistent across various years. The authors hypothesised that the attractive effect of verbenone only occurs at low population densities and disappears at higher population densities (Rappaport et al. 2000). *Orthotomicus erosus* was attracted by high amounts of verbenone (40 mg/d), and *I. sexdentatus* might be attracted to moderate amounts of verbenone (release rate of 2 mg/d) (Etchebeste et al. 2013), similar to what has been proposed for *D. frontalis* (Rudinsky 1973). The attraction of *Ips grandicollis* to baited traps was more than doubled by verbenone with a release rate of 40 mg/d, but not to verbenone-containing traps stapled to host trees without bait (Dodds and Miller 2010). Also, *I. typographus* was attracted to high release rates of verbenone (93 mg/d) in the presence of host kairomones, even though the overall number of attacks per tree was low (Niemeyer et al. 1995).

Actual ‘repellence’ has been reported even less, but this is presumably also because beetle catch numbers are typically extremely low when passive traps are used without any bait (Reeve and Strom 2004): the addition of a repellent cannot decrease the trap catches any further, unless an extremely large sample size is used that allows to statistically differentiate the low trap catches of blank traps to even lower catches in traps with repellents. For example, no repellent effect was observed in *T. yunnanensis* and *T. minor* using an olfactory walking assay in the laboratory (Wu et al. 2019) and in *D. brevicornis* and *I. sexdentatus* in

the field (Etxebeste and Pajares 2011; Fettig et al. 2012a). VanDerLaan and Ginzl (2013) tested verbenone without additional compounds and thus caught fewer individuals of the target species *X. germanus* and *X. crassiusculus* than in non-baited control traps. This is so far the only study providing evidence for a repellent effect of verbenone that is not attraction inhibition in the field. Similarly, in walking assays, the coffee berry borer *Hypothenemus hampei* had a significant preference for the solvent-only treatment when exposed to verbenone (Jaramillo et al. 2013). Technically, in studies where only inhibition is tested (i.e. verbenone tested against an attractive blend), repellence—if present—cannot be detected since it would be covered by the inhibitory effect. Therefore, for many species it is not known whether verbenone may also be a repellent that acts as an inhibitor when presented together with attractants (see Fig. 5-3 options a, b). This difference, however, is important if we want to understand whether verbenone also has an effect in the absence of attractive compounds as assumed to be the case at later stages of an attack, when the release of aggregation pheromones expires (Bakke 1981; Zhang et al. 2000a, b; Pureswaran and Sullivan 2012). It has also direct implications for semiochemical-based management approaches because this knowledge helps to identify scenarios during which the application of the inhibitory verbenone is efficacious and when not.

The majority of behavioural studies addressed flight response (conducted with baited traps), and comparatively few investigated walking behaviour or host colonisation (including host finding, host acceptance, oviposition and feeding; Table 2) under the influence of verbenone. In case more than one behaviour was studied for a species, the response is typically coherent in the sense that the inhibitory effect of verbenone affects the behaviour at different levels (e.g. Byers and Wood 1980, 1981; Ryker and Yandell 1983; Byers et al. 1989; Hayes et al. 1994; Zhang et al. 1999; Zhang et al. 2006; López et al. 2013; Zhao et al. 2017; Wu et al. 2019; and also see Table 2). Concordantly, verbenone did not affect the ratio of landing vs. attacking *D. brevicomis* (Bertram and Paine 1994a). Other effects of verbenone have rarely been tested, but point in a similar direction. Walking *D. ponderosae* showed arresting behaviour in proximity to their aggregation pheromones less frequently in the presence of verbenone (Ryker and Yandell 1983). In laboratory assays with walking *I. paraconfusus*, increasing release rates of (+)-verbenone over four orders of magnitude caused declining proportions of both sexes to reach the source of their aggregation pheromone components (Byers and Wood 1981). Inhibition of colonisation might also be due to a feeding deterrence by verbenone in at least some species: verbenone had no effect on the frequency of incomplete galleries of *I. sexdentatus* and *O. erosus* (Etxebeste et al. 2013), but it seemed to slow down the boring activity of male *I. pini*

(Sallé and Raffa 2007). Feeding performance and acceptance of artificial diet were also reduced in *I. typographus* even to a higher degree than was observed for  $\alpha$ -pinene (Facciolli et al. 2005), a major compound of tree defence in conifers. In fact, also the toxicity of verbenone to bark beetles was higher than that of  $\alpha$ -pinene (Sallé and Raffa 2007). This is surprising given the fact that the conversion of  $\alpha$ -pinene is commonly regarded as detoxification to improve the quality of the brood habitat (Hunt and Borden 1989; Lieutier 2004). Nevertheless, the toxicity of verbenone is still considerably lower compared to neurotoxins used as conventional insecticides (Rivera-Davila et al. 2021).

The beetle response to their pheromones depends on the context, e.g. it can vary between generations and throughout the season (Heber et al. 2021) and also depends on the nutritional status of an individual (Bennett and Borden 1971; Němec et al. 1993). While it is obvious that verbenone conveys relevant information to dispersing beetles, it is, however, easy to imagine that fatigued individuals may be obliged to land on a tree that is rather unattractive due to high verbenone emissions and accept a low chance of reproduction if the only alternative is incipient exhaustion and death. Analysis of the nutritional state of this proportion of individuals that land in verbenone-baited traps could shed some light on the importance of bark beetle physiology. Altogether, we still know very little about why some individuals within a population are indifferent to verbenone (Fig. 5-3c) and why these proportions vary strongly among different studies, years, and sites. Identification of these drivers will enhance our understanding of the fascinating ecology of scolytine beetles, but will also help to make the practical application of verbenone as a semiochemical for pest management more effective.

### Dose dependency and synergistic inhibition

Many of the behavioural studies on the response of bark beetles to verbenone are driven by a strongly applied background. Therefore, they often address the questions on effective doses or relevant concentrations needed to affect beetle behaviour. The inhibitory effect of verbenone on baited traps in the field seems to be positively correlated with the verbenone dosage in general (but see Paine and Hanlon 1991; Erbilgin et al. 2008; López et al. 2013; Byers et al. 2018, 2020). This relation can be linear (Schlyter et al. 1989) but also log-linear (Lindgren and Miller 2002b), log–log like (Tilden and Bedard 1988; Miller et al. 1995; Lindgren and Miller 2002b) or a kinetic decay function of second order (Byers et al. 2020). Most frequently, response thresholds were described (Ryker and Yandell 1983; Schlyter et al. 1989; Bertram and Paine 1994b; Devlin and Borden 1994; Rappaport et al. 2000; Lindgren and Miller 2002a; Fettig et al. 2007; López et al. 2013; Audley et al. 2020), even

though this could in some cases be an artefact of sample size and/or unlucky choice of verbenone release rates that lead to the observation of an ‘all-or-nothing’ response threshold. The results from field trapping experiments agree with what was found on the colonisation behaviour, i.e. stronger inhibition with higher amounts of verbenone (Salom et al. 1997; Borden et al. 2003; Bentz et al. 2005; Schiebe et al. 2011; Etxebeste et al. 2013; Byers et al. 2020; Fettig and Munson 2020), but with some exceptions that show a consistent inhibitory effect for various dosages that were tested (Bakke 1987; Safranyik et al. 1992; Devlin and Borden 1994; Fettig et al. 2020).

Some volatiles from other sources including non-host species (and therefore generally referred to as ‘non-host volatiles’, NHV) can also inhibit bark beetle aggregation (Byers et al. 1998, 2004; Zhang and Schlyter 2004; Huber et al. 2020). There is ample evidence across many bark beetle genera, that blends containing various NHVs and verbenone elicit stronger inhibition than each compound alone (e.g. Zhang et al. 2001; Fettig et al. 2005, 2009b; Shepherd et al. 2007; Shepherd and Sullivan 2013; Unelius et al. 2014), suggesting that there is some kind of synergism in attraction inhibition. Even if verbenone and volatiles emitted from plants have different origins and thus ecological meanings, the integration of different stimuli can sometimes lead to a modified behavioural response compared to the stimulus of a single compound (Andersson et al. 2010). For this literature analysis, verbenone was considered a synergistic inhibitor (Table 2), if a blend containing verbenone was inhibitory compared to bait-only controls, regardless of the effect of each individual blend component. From a practical point of view, verbenone and other volatile compounds are often used together in blends to optimise attraction inhibition for tree protection (e.g. Zhang et al. 2000a, b; Zhang and Schlyter 2004; Unelius et al. 2014; Huber et al. 2020).

Ratios of inhibitors vs. attractants might be more relevant than absolute amounts (Bentz et al. 2005). Byers et al. (2020) investigated the effect of relative verbenone concentration compared to the attractant quercivorol in *E. fornicatus* and found a sigmoidal positive relationship between verbenone release rate and reduction in attraction. This might explain why verbenone occurs at the early stages of colonisation (see Q. 1.2), but without inhibiting attraction at this stage. However, also in studies in which variable absolute amounts of verbenone were tested, the concentrations of the attractants were typically not adapted to the varying verbenone concentrations. Thus, verbenone-to-attractant ratios are not stable, and in fact relative amounts are tested. Bertram and Paine (1994b) conducted the only study to disentangle absolute and relative release rates and found that for *D. brevicomis*, absolute release rates were more important than ratios. They hypothesised that this could be due to different compound-specific detection thresholds and supports

their independent perception and additive effects. Finally, since natural variation in levels of attractive compounds may underlie lower variation compared to inhibitory signals (J.A. Byers, pers. comm.), ratios of attractant-to-inhibitor may matter for some lower concentrations, while extremely high amounts of an inhibitor may be effective regardless of the level of attraction.

## Ecological implications

### Intraspecific signalling and sex-specific differences

Like most of the aggregation pheromone components that attract both sexes of gregarious bark beetle species to mediate the attack on hosts, there is ample evidence that also the inhibitory effect of verbenone affects both sexes, for example in *D. brevicomis* (Bedard et al. 1980; Hayes and Strom 1994; Strom et al. 2001; Fettig et al. 2005, 2009a; Erbilgin et al. 2007, 2008), *D. ponderosae* (Miller et al. 1995; Borden et al. 1998; Fettig et al. 2012b) and *D. valens* (Zhang et al. 2006; Fettig et al. 2007), but possibly not in *D. frontalis* (Salom et al. 1992; Hayes et al. 1994) and *D. rhizophagous* (Cano-Ramírez et al. 2012) in which males showed a greater response. The latter case also seems to be typical for some *Ips* species. Male *I. typographus* generally seem to react more strongly to inhibitory compounds (Byers 1993), including verbenone in the context of baited traps (Schlyter et al. 1989; Zhang and Schlyter 2003; Unelius et al. 2014; but contrary to our pers. obs. in the field). Male response to verbenone-baited traps was also more strongly inhibited in *I. duplicatus* (Zhang et al. 2001). The opposite was the case in a multi-year field study on trap logs (Bakke 1981). Also in *I. paraconfusus*, *I. latidens* and *I. pini* females reacted somewhat more strongly to verbenone or no difference between the sexes was observed (Byers and Wood 1980, 1981; Miller et al. 1995). Furthermore, the frequencies of ORN classes in *I. typographus* do not appear to differ between sexes (Andersson et al. 2009) and this is also valid for the ORN class that detects verbenone (M.N. Andersson, pers. obs.). Furthermore, no sex difference was found in response to the spatial separation of the aggregation pheromone and verbenone (Binyameen et al. 2014). These findings suggest that a stronger inhibition of males in some *Ips* species in response to verbenone is likely not due to a higher olfactory sensitivity to the compound.

Altogether, one of the differences between the monogamous genus *Dendroctonus* and the polygynous genus *Ips* may be, that in the latter males react somewhat stronger to inhibitory compounds. One might expect that it is more crucial for the colonising sex to discern suitable breeding sites. In many other insects where females choose the oviposition site, they generally show a stronger response than males to host plant volatiles (Szendrei and

Rodriguez-Saona 2010). On the other hand, as verbenone biosynthesis is rather associated with male bark beetles (Q1.1) verbenone could represent a general signal for male rivalry (Rudinsky et al. 1974) to avoid competition among male conspecifics. According to Kirkendall (1983), female-initiated monogyny is the ancient mating system of bark and ambrosia beetles. The later joining male has an evolutionary benefit by preventing the mating of the female with a second male ('guarding', Kirkendall 1983) and habitat competition by further colonising females. Nevertheless, also females should avoid competition from other egg-laying individuals, as their offspring compete for space and food (Byers and Wood 1980; Byers et al. 1984; Anderbrant et al. 1985). The quantitative analysis of life history traits likewise suggests that the mating system of a species is not strongly associated with its response to verbenone (Fig. 4, e.g. inhibition occurred in  $17/26 \approx 65\%$  of monogamous,  $9/12 \approx 75\%$  of polygamous and  $12/15 \approx 80\%$  of inbreeding species;  $p = 0.14$ ). In fact, the mating system seems to be of low relevance to predict a species' response to verbenone compared to its host use strategy (Cramér's V: 0.19 vs. 0.24, Fig. 4; but, for example, only for six cone/seed feeding species a response to verbenone was described), at least for the pool of species for which a behavioural response to verbenone has been described so far ( $N = 53$ ). The common response to verbenone may also be conserved even in genera like *Ips* that has evolved polygynous mating systems. To test this hypothesis, the evolutionary origins of verbenone receptors in various species of bark and ambrosia beetles need to be investigated. Ultimately, sex-specific density regulation may also be caused by other compounds that guide female site selection, e.g. *trans*-verbenol in *D. brevicomis* (Byers 1983a, 1984).

Nevertheless, it is noteworthy that verbenone amounts associated with *I. typographus* and *I. paraconfusus* are relatively low, while for *D. brevicomis* and *D. frontalis* enormously large amounts were found (Q1.2). *Ips typographus* is assumed to require a comparatively high infestation density when colonising living trees—more than twice the necessary colonisation density as needed for *D. brevicomis* (Raffa 2001). It could be the reason why *I. typographus* does not release large amounts of verbenone and suggests that a species-specific interpretation is needed, despite the commonly shared inhibitory effect of verbenone. The differences between sexes in the response to verbenone observed for some scolytine species can also be dose-dependent (López et al. 2013; Zhao et al. 2017; Audley et al. 2020) and are generally never pronounced: there is no report that one sex remained completely unaffected by inhibitory compounds. In conclusion, it therefore seems that verbenone is a more general indicator of high feeding activity of Scolytinae affecting both sexes to some degree to reduce intraspecific

competition. At this point, a species-specific perspective is required to characterise the function of verbenone for intraspecific communication and define whether verbenone is a passive cue or an actual signal in a given species.

To assess the ecological function of verbenone, also the spatial range of its effect needs to be considered. It has often been observed that verbenone treatments reduce both the risk of infestation for single trees and the colonisation density within a tree at the same time (Lindgren et al. 1989; Safranyik et al. 1992, and pers. obs.; but see Devlin and Borden 1994). Verbenone did not affect the ratio of landing vs. successfully colonising *D. brevicomis*, but caused a reduced colonisation density (Bertram and Paine 1994a), suggesting its function as a wide-range inhibitor, i.e. to mediate the colonisation among trees, but not within a tree. For the same species, it was observed that their resting behaviour next to the aggregation pheromone was less frequent in the presence of verbenone (Ryker and Yandell 1983), underlining its function as a short-range inhibitor. The 'active inhibitory range' (AIR) can be defined as the maximum distance at which a significant change in beetle behaviour can still be observed compared to untreated controls (Zhang and Schlyter 2003). Most field studies suggest an AIR of verbenone of only a few metres (Shore et al. 1992; Niemeyer et al. 1995; Huber and Borden 2001; Jakuš et al. 2003; Zhang and Schlyter 2003; Ranger et al. 2013; Fettig et al. 2015; Byers et al. 2018, 2020), only a few report inhibition between 10 and 15 m (Mafra-Neto et al. 2014). Concordantly, grid distances of verbenone point sources typically lie between 5 and 9 m in studies where verbenone is tested to inhibit aggregation within a whole area (Borden et al. 1992; Devlin and Borden 1994; Fettig and Munson 2020; Fettig et al. 2020) or 10–15 m (Amman et al. 1989; Lindgren et al. 1989; Devlin and Borden 1994; Borden et al. 2003, 2006; Progar 2005). For some ambrosia beetle species, however, the AIR might be below 1 m, depending on the rate of verbenone release (Byers et al. 2020; Rivera et al. 2020). This might be due to their low tendency to show a gregarious behaviour (Hulcr and Stelinski 2017) and suggests that verbenone might have a greater importance for bark beetles as a short-range inhibitor to mediate spacing within a tree and thereby limit intraspecific competition (Byers et al. 1984). Given that the applied verbenone dosages are often unnaturally high (Bertram and Paine 1994a; McPherson et al. 1997; Pureswaran and Sullivan 2012), the observed AIR could be overestimated compared to natural conditions. This would at least not necessarily contradict the interpretation of verbenone as a short-range inhibitor with an AIR of 1 m or less. Bentz et al. (1997) did not measure pheromone contents, but investigated the attack dynamics of *D. ponderosae* around a focal tree that was baited for 24 h. They found that attacks continued on an already infested tree, while neighbouring trees were already under attack before the focal tree was saturated.



They concluded that the inhibitory effect of verbenone rather lies in the very short range, below one metre, and an attack shift to an adjacent tree in this species is most likely caused by a spread of the aggregation pheromone plume increasing the attractiveness of not yet attacked trees. However, actual verbenone concentrations were not measured in this study. Byers (1984, 1992b) reported a species-specific, minimum distance of spacing apart attack holes for several primary bark beetle species. He likewise suggested that in some species aggregation pheromones and verbenone may play a role in spacing colonisation sites within a tree and allow the reduction in intraspecific competition in the phloem layer.

### Interspecific signalling and trophic relationships

Verbenone seems to be ubiquitous among Scolytinae. Thus, the question arises whether verbenone might also help to avoid competition between sympatric bark and ambrosia beetle species. Most studies indicate that this is to the benefit of all competing species, rather than imposing an advantage for only a single species. For example, the commonly co-occurring species *I. typographus* and *Pityogenes chalcographus* are both inhibited by verbenone (Bakke 1987; Byers 1993), which is consistent with the fact that their interaction is not strictly negative despite an overlap in habitat preference (Göthlin et al. 2000; Schebeck et al. 2023, and pers. obs. on windfelled Norway spruce). Furthermore, three sympatric pine-feeding species *I. latidens*, *I. pini* and *D. ponderosae* were equally inhibited by verbenone (Miller et al. 1995), and likewise *D. ponderosae* and *D. brevicomis* (Hayes and Strom 1994). Inhibition by verbenone seems also common among sympatric ambrosia beetle species (Werle et al. 2019; Rivera et al. 2020). This common response is not surprising, since also attractive pheromone components are sometimes shared among species (Pureswaran et al. 2008).

The hypothesis that verbenone is a general cue to avoid interspecific competition is also supported by the finding that the enantiomeric composition of verbenone usually does not have a great effect on the species' response (Bakke 1981; Paine and Hanlon 1991; Zhang et al. 2006; Byers et al. 2018) and thus is generic. In addition, the enantiomeric composition of verbenone within a species is rather variable (Q1.1), corroborating its general nature. In a complex olfactory landscape with high levels of background odours (Byers et al. 1984, 2000; Byers 2004; Raffa 2001), this flexibility can help to reliably avoid competition among numerous bark and ambrosia beetles using the same habitat, but also allows secondary species to detect suitable habitats.

Even if verbenone is informative for many sympatric scolytine species and causes the same behavioural response, it may still be possible that some species can exploit this signal to their advantage. Wu et al. (2019) found that both sexes of the sympatric species *T. minor* and *T. yunnanensis*

are inhibited by verbenone. However, they observed that verbenone biosynthesis was highest in female *T. minor* and concluded that interspecific competition was regulated by the colonising sex of the less aggressive species. Finally, slight differences in concentration and release rate, probably related to differences in detection thresholds, may further affect intraspecific competition. For example, *O. erosus* was attracted to high release rates of verbenone, while verbenone at the same release rate caused inhibition in *I. sexdentatus*, and this was the opposite for low release rates (Etxebeste et al. 2013). Given that in many studies with a focus on practical application, rather unnaturally high release rates of verbenone were used (Bertram and Paine 1994a; McPherson et al. 1997; Pureswaran and Sullivan 2012), thus, fine-tuned and species-specific differences in the response to verbenone to regulate interspecific competition might have remained undetected.

As verbenone is common among Scolytinae, also bark beetle-associated organisms might be able to detect and respond to that cue. Predators, like *Thanasimus* spp., can detect (Hansen 1983) and respond to aggregation pheromone components of their bark beetle prey (Schlyter et al. 1989; Pureswaran et al. 2008; Heber et al. 2021). At least *Thanasimus formicarius* possesses neurons specific to (–)-verbenone (Tømmerås 1985), which elicits strong antennal responses with low thresholds (Hansen 1983). Surprisingly, bark beetle predators are not generally attracted by verbenone, even though this interaction has been intensely studied, particularly for the two genera *Thanasimus* (Col., Cleridae) and *Temnochila* (Col., Trogossitidae). Even contrary to the expectations, verbenone inhibits the attraction of *T. formicarius* to bark beetle kairomones (Etxebeste and Pajares 2011; Etxebeste et al. 2013). Also *Thanasiumus dubius* is either inhibited by (Strom et al. 1999; de Groot and DeBarr 2000) or indifferent to (Payne et al. 1992; Hayes et al. 1994) the addition of verbenone to bark beetle pheromone baited traps. The response of *Temnochila chlorodia* is rather variable (Gillette et al. 2009) and might depend on the release rate. A relatively high release rate of 50 mg/d increased the attraction for that species to host volatiles with and without bark beetle kairomones (Fettig et al. 2007) and a low release rate of only 2.5 mg/d was inhibitory (Erbilgin et al. 2007), as well as intermediate release rates of 8–12 mg/d (Hayes and Strom 1994; Strom et al. 2001). Also, *Temnochila caerulea* showed a negative response to verbenone (Etxebeste and Pajares 2011). In a field trapping assay on multiple target species, *Thanasiumus undulates*, *Enoclerus sphegeus*, *Enoclerus lecontei* and *Lasconotus complex* were inhibited, while *Lasconotus subcostulatus* was not affected (Lindgren and Miller 2002a). The attraction of another bark beetle predator, *Corticeus praetermissus*, was not consistent across different experiments (Lindgren and Miller 2002a). The authors suggested that predators and parasitoids, that either depend

on the presence of adult bark beetles or larvae at an early stage of development, may also avoid brood systems of a certain age and thus negatively react to verbenone. An eight-component blend containing verbenone was an oviposition stimulant for *Rhizophagus grandis* in a no-choice experiment on larvae from *D. valens* and *D. micans* in an artificial phloem medium (Grégoire et al. 1991).

Altogether, the evidence that predators of bark and ambrosia beetles are attracted to verbenone seems rather weak. This might be explained by the fact that the predators that are considered in these studies (mostly *Thanasimus* spp. and *Temnochila* spp.) are typically caught in low numbers compared to their bark beetle prey, making it difficult to detect significant trends with the same sample size otherwise used for gregarious bark beetles. Second, verbenone alone might not be attractive for these natural enemies, but require the presence of additional yet unknown cues. It would be possible that natural enemies only have specific periods during which they are susceptible to prey kairomones, while at other times they are indifferent, or exhibit seasonal fluctuations in abundance, making it difficult to study attraction (e.g. see Hayes and Strom 1994; Lindgren and Miller 2002a). There are a large number of natural enemies that prey on adult bark beetles or their larvae from diverse taxonomic groups including parasitoids from Hymenoptera, Diptera, or Acari (Wegensteiner et al. 2015), but these groups have so far received no attention regarding their response to verbenone. This, however, would be an interesting and important aspect to assess the efficacy of verbenone treatments to prevent bark beetle attacks in forest protection.

## Conclusions

The antennal detection of and the response to verbenone seems to be well conserved in the highly evolved pheromone system of bark and ambrosia beetles and may thus represent an ancestral state. Verbenone is an attraction inhibitor for at least 38 scolytine species from 16 genera, i.e. 73% of the species for which the behavioural response has been tested, representing < 1% of the existing bark and ambrosia beetle species (Hulcr et al. 2015). An exception to this inhibitory effect may be found in strongly secondary species, which are more often either indifferent to or even attracted by verbenone. Attraction was also reported for a few primary species for specific release rates or during specific phases in the population dynamics. The host usage strategy (i.e. primary vs. secondary or cone/seed feeding) was more strongly related to a species' response to verbenone than the mating system of a species. While many sympatric bark beetle species are commonly inhibited by verbenone, it is surprising that natural enemies are not consistently attracted by verbenone. More research should be invested in studying the

effect of verbenone on other groups of natural enemies, such as parasitoid wasps or predatory flies.

Even if inhibition by verbenone seems most common among bark beetles, one should keep in mind that the most frequently studied taxa are not necessarily good representatives for the extraordinarily high diversity of lifestyles within the Scolytinae and so the description of verbenone as a 'universal bark beetle repellent' can be misleading. The term 'inhibitor' should be preferred over the term 'repellent' because that is what has been tested and proved in most field trapping studies, even though in pest management applications the latter is traditionally more common. Even if verbenone is an inhibitor to numerous species, its precise function may differ among species and vary according to the actual source, the timing of synthesis, sex-specific detection and response thresholds and the resulting spatial range of inhibition, which are not well studied for most species. Most importantly, we lack studies on atmospheric verbenone concentrations around infested trees as most knowledge about pheromone amounts stems from gut extracts, but we do not know how these concentrations relate to actual emissions.

The analysis of more than 200 studies suggests that verbenone can be interpreted as an intra- and interspecific inhibitor of sympatric Scolytinae, most likely to prevent overcrowding on densely colonised hosts. However, results from studies on the temporal patterns of verbenone synthesis often contradict the assumption that verbenone is a compound that is released from adult beetles at later stages to terminate colonisation for the avoidance of intra- and interspecific competition. Instead, relatively large amounts can be found much earlier, for example, in newly emerged beetles, too early to deter mates that are still needed to overwhelm tree defence and mating at this stage. There is a discrepancy, however, between studies on extracts vs. headspace measurements, stressing the importance of verbenone sources outside the beetles.

An increase in verbenone amounts at later stages of colonisation may primarily be caused by microbial processes outside the beetles' bodies. At least for many species, instead of a pheromone mediating anti-aggregation, verbenone may rather be seen as a passive by-product of intense feeding activity on  $\alpha$ -pinene-rich substrate. Bark beetles and their associated microorganisms help to accelerate the oxidation to the final product verbenone. The fact that probably a substantial proportion of this microbial biotransformation of verbenol to verbenone occurs outside the beetles' body also contradicts the classification of verbenone as an actual pheromone, or requires the perception of beetles as a holobiont (Six 2013) as a more explanatory ecological unit. Nevertheless, a bark beetle species-specific interpretation is necessary to decide whether verbenone can be considered a pheromone of the bark beetle holobiont or whether it is rather described as an allelochemical. Altogether, it is likely that verbenone is

**Table 4** Summary of the main findings of this literature review and implications for future studies on verbenone and verbenone-based approaches within integrated bark beetle management strategies

| Finding  | Remaining questions for future research   | Implications/benefits for bark beetle management with verbenone   |
|--|---|---|
| <p>Verbenone is a common compound in the context of scolytine beetles and can typically be detected by their neuronal system (but less than 1% of the species have been studied so far)</p> <p>Verbenone is most likely synthesised by associated microorganisms in the guts or outside the beetles' bodies and thus a passive cue in most species, but this may differ among species; for a functional understanding of the semiochemical communication of bark beetles, the concept of a 'holobiont' (i.e. the entity of the bark beetle with its associated microorganisms) is proposed</p> | <p>Can bark beetles (or at least some species) actively control the amount of verbenone that is released?</p> <p>How do gut concentrations of verbenone relate to emitted amounts and release rates from an infested site?</p> <p>Which organisms are involved?</p> <p>What are underlying pathways?</p>                      | <p>Artificial release of verbenone may affect a broad range of sympatric scolytine species</p> <p>A thorough understanding of the ecological backgrounds will help to identify scenarios when verbenone application is most efficacious (or more importantly, when it is not)</p>   |
| <p>Most of the species (at least 38; from 16 genera) are inhibited by verbenone, at least for high release rates (1–100 mg/d), excluding at least some secondary species, as well as some cone/seed feeders; the life history trait host usage strategy was associated more with a species' response to verbenone than the mating system</p>   | <p>Is verbenone also repellent or is the presence of an attractant necessary to induce a change in behaviour?</p> <p>What are the (physiological) reasons that verbenone does not result in an inhibition in all individuals of a population?</p> <p>Evolution and evolutionary drivers of verbenone response strategies?</p> | <p>If verbenone is used to protect trees from bark beetle infestation, the treatment likely targets a broad range of economically relevant species</p> <p>Knowing if verbenone alone is also repellent without the presence of an attractant will help to identify suitable application scenarios</p> <p>Identification of the reasons why some individuals of a population ignore verbenone will likewise help to identify scenarios when verbenone application is most efficacious (or more importantly when it is not)</p> |
| <p>Natural release rates of verbenone from naturally infested trees are not known (except two estimations)</p>   | <p>What atmospheric concentrations of verbenone can be expected from natural infestations by different bark beetle species?</p>   | <p>Knowledge about natural verbenone concentrations will help to optimise verbenone blends and thus make bark beetle management more effective and cost efficient</p>   |
| <p>Natural enemies are not consistently attracted to verbenone, but the behavioural responses of only a few coleopteran taxa (<i>Thanasimus</i>, <i>Thamnochila</i>) have been studied</p>   | <p>What is the behavioural response of other natural enemies (e.g. parasitoids wasps and predatory flies) to verbenone?</p>   | <p>Artificial release of verbenone will likely not affect the predator/prey ratio so that bark beetle population management will not benefit from an increase in natural enemies</p> <p>Natural enemies that are attracted by verbenone may help to increase the effectivity of verbenone application by targeting those beetles that ignored the verbenone treatment</p>   |

a passive cue rather than an active signal in most species, but it remains open whether the beetles can actively control the amounts and the time point of verbenone release in response to intraspecific competition.

Filling these gaps in a more than 50-year-old field of research (Table 4) will on one hand improve our ecological understanding of how tiny bark beetles manage to organise their attacks to mass aggregate on suitable hosts—something, still not possible to predict—and can have effects so large that they become visible on a landscape scale. On the other hand, making use of inhibitory substances is an interesting, environmentally friendly approach to manage bark beetle populations in economically used forests and ambrosia beetles in horticultural tree crops (Table 4). While aggregation pheromones are routinely used for monitoring purposes, inhibition of mass aggregation with semiochemicals still comes with some inherent uncertainty. Despite available technologies, semiochemical-based tools are comparatively rarely used in agriculture and forestry so far, even though conventional insecticides as the historically only alternative decrease in acceptance due to their environmental, social and human health impact, so that more sustainable alternatives are urgently needed (Gillette and Fettig 2021; Mafra-Neto et al. 2022). A better understanding of the species-specific roles of verbenone, including knowledge about actual verbenone release rates from infested trees, the active or non-active participation of the beetles in verbenone release, intrinsic and extrinsic factors that cause beetle individuals to not respond to verbenone and the effects on natural enemies of bark beetles can help to optimise sustainable verbenone-based semiochemical management tools within the integrated management of pest populations.

## Author contributions

TF developed the research question, conducted the literature search, summarised the literature, drafted, wrote, and revised the manuscript. MS, TB, and PHWB contributed to the overall structure of the manuscript and especially to the ecological interpretations. MNA, GH, and JK contributed to the content and its interpretation. PHWB helped to develop the idea and direction of this review at the very beginning. HD and TB initiated the project and together with PHWB provided guidance throughout the study. All authors critically revised the manuscript and approved the final version.

**Acknowledgements** We want to thank Sebastian Pollok and Anna-Mareike Meier for their help in literature research. We thank Anita Kiesel for providing the picture of a spruce bark beetle and Fenris Måling for drafting, realisation, and assembly of several figures. We also thank two anonymous reviewers for their constructive feedback that helped to improve the manuscript. The project is funded by the German Federal Ministry of Food and Agriculture (BMEL) through the

Agency of Renewable Resources (FNR) within the funding program ‘Renewable Resources’ and according to a decision of the German Parliament (FKZ 22017117 and 22012918). MNA was funded by the Swedish Research Council FORMAS (grant #2018-01444) and the Foundation in Memory of Oscar and Lili Lamm. PHWB acknowledges funding by the German Research Foundation (DFG) (Emmy Noether grant number BI 1956/1-1). MS was financially supported by the Austrian Federal Ministry for Agriculture, Forestry, Regions and Water Management, Waldfonds projects ‘NewIPS’ (project number: 101686) and ‘IpsEMAN’ (project number: 101687).

**Funding** Open Access funding enabled and organized by Projekt DEAL. The project is funded by the German Federal Ministry of Food and Agriculture (BMEL) through the Agency of Renewable Resources (FNR) within the funding program ‘Renewable Resources’ and according to a decision of the German Parliament (FKZ 22017117 and 22012918). MNA was funded by the Swedish Research Council FORMAS (grant #2018-01444) and the Foundation in Memory of Oscar and Lili Lamm and PHWB was funded by the German Research Foundation (DFG) (Emmy Noether grant number BI 1956/1-1). MS was financially supported by the Austrian Federal Ministry for Agriculture, Forestry, Regions and Water Management, Waldfonds projects ‘NewIPS’ (project number: 101686) and ‘IpsEMAN’ (project number: 101687).

**Availability of data and materials** The data set and R code generated and analysed during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interests** The authors declare no financial or non-financial conflict of interest.

**Ethical approval** This statement is not applicable.

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