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Odours attracting *Medetera* (Diptera) predators to spruce bark beetles in a multitrophic system

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Cover: Dispersal of bark beetles *I. typographus* and adult *Medetera* flies in conifer forests. (Illustration: Zehrina Karic and Maria Sousa)

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Abstract

Tree-killing bark beetles cause great economic and ecological damage worldwide. Several long-legged fly species of the genus *Medetera* are natural enemies of tree-killing bark beetles and are of interest as potential biological control agents. However, flies within the *Medetera* genus have been poorly studied to date, partly due to very difficult species identification that often requires studying the fine structures of male genital morphology. In this thesis, morphological analysis and barcoding were applied in species level identification of different *Medetera* specimens collected from the bark of Norway spruce trees in Southern Sweden infested with the Eurasian spruce bark beetle, *Ips typographus*. Adult *Medetera* flies use olfaction to locate bark beetle-infested trees. Olfactory cues (odours) are detected by various types of hair-like structures called sensilla distributed on the fly antennae and maxillary palps. This thesis shows that the number and subtypes of sensilla differs between *M. signaticornis* and *M. infumata*, the two most common species found at the study sites. Therefore, it is possible that these two species respond to different odours, or process the same odours in different ways.

Further analysis showed that male and female *M. signaticornis* were able to detect more than 20 compounds emitted from Norway spruce trees infested with *I. typographus*. These compounds included metabolites produced by the trees and also compounds produced by bark beetles and associated microorganisms. Such detection of odours from different trophic levels by *Medetera* flies may facilitate the location of infested trees throughout a bark beetle attack. Multiple comparisons of synthetic blends containing different combinations of these compounds demonstrated that some compounds (*e.g.* (–)-*cis*-verbenol, ipsdienol, myrtenol, α- and γ-terpinene; limonene, camphor, terpinen-4-ol and borneol) were more important than others in the attraction of *Medetera* species. Overall, the findings presented in this thesis can facilitate identification and monitoring of *Medetera* flies and might help to improve management of *I. typographus* in the future.

Keywords: bark beetle management, biological control, chemical ecology, forestry, host associations, morphology, multitrophic interactions, natural enemies, odours, synthetic blends.

Dedication

To Catarina, Sebastian and João.

"*The only impossible journey is the one you never begin*"

Tony Robbin

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Sousa, M.**, Karlsson, Green K., Auger-Rozenberg, A.M., Pollet, M., Birgersson, G., Becher, P.G. & Bras, A. Exploring identity and diet range of species within the genus *Medetera* that are important natural enemies of tree-killing Scolytinae. Manuscript.

II. **Sousa, M.**, Ignell, R., Pollet, M., Green, K.K., Becher, P.G. & Birgersson, G. (2023). Antennal and maxillary palp morphology, and sensillar equipment, of the spruce bark beetle predators, *Medetera signaticorni*s and *Medetera infumata* (Diptera: Dolichopodidae). *Arthropod Structure & Development 72*, 101229.

III. **Sousa, M.**, Birgersson, G., Karlsson Green, K., Pollet, M. & Becher, P.G. (2023). Odors attracting the long-legged predator *Medetera signaticornis* Loew to *Ips typographus* L. infested Norway spruce trees. *Journal of Chemical Ecology*. In press, published online.

IV. **Sousa, M.**, Andersson, A., Englund, J.E., Flöhr, A., Pollet, M., Karlsson Green, K., Birgersson, G. & Becher, P.G. Attractiveness of synthetic odour compound blends for the tree-killing beetle *Ips typographus* and predatory flies of the genus *Medetera*. Manuscript.

The contribution of Maria Sousa to the papers included in this thesis was as follows:

I. Designed the study together with the co-authors. Collected the insects, extracted DNA from the specimens and analysed the data. Wrote the manuscript with input from the co-authors.

II. Collected the insects, created SEM pictures to study the morphology of insect olfactory organs and analysed the data. Wrote the manuscript with input from the co-authors.

III. Designed the study with the co-authors. Collected headspace samples and insects in the field, performed GC-MS and GC-EAD experiments, identified active compounds and analysed the data statistically. Wrote the manuscript with input from the co-authors.

IV. Designed the synthetic blends and field experiments together with the co-authors. Performed data collection and data analysis. Wrote the manuscript with input from the co-authors.

Publications not included in this thesis

- **Sousa, M**., Mulaosmanovic, E., Erdei, A.L., Bengtsson, M., Witzgall, P. & Alsanius, B.W. (2023) Volatilomes reveal specific signatures for contamination of leafy vegetables with *Escherichia coli* O157:H7. Food Control, 109513. https://doi.org/10.1016/j.foodcont.2022.109513

- **Sousa, M**. (2019) On the multitrophic interactions between *Ips typographus* their tree host, associated microorganisms, and a predatory *Medetera* fly. Introductory Research Paper. Institutionen för Växtskyddsbiologi, Sveriges Lantbruksuniversitet

- Mulaosmanovic, E., Farkas, S., Vågsholm, I., Darlison, J., **Sousa, M**., Mogren, L., Gharaie, S. & Alsanius, B.W. (2019). Safety risks associated with dispersal of *E. coli* O157: H7 in home sprouting modules. LWT, 101:783-788. https://doi.org/10.1016/j.lwt.2018.11.086

Abbreviations

BHD	Breast height diameter
COI	Cytochrome oxidase subunit I
GC-EAD	Combined gas chromatography and electroantennographic detection
GC-MS	Combined gas chromatography and mass spectrometry
VOC	Volatile organic compounds
SEM	Scanning electron microscopy

1. Introduction

Insect pests kill millions of hectares of forests around the world every year (Raffa *et al.*, 2008; Seidl *et al*., 2014). In conifer forests, the species generally known to cause the most damage are bark beetle species of the genera *Dendroctonus* and *Ips* (Fettig *et al*., 2022; Wermelinger & Jakoby, 2022). In Europe, the Eurasian spruce bark beetle, *Ips typographus* L. (Coleoptera: Scolytinae), is becoming an increasingly serious pest of Norway spruce (*Picea abies* L. Karst.). In the years 2002 and 2010, *I. typographus* killed around 14 million m^3 of spruce, while by 2018 the number of killed trees had significantly increased, to more than 40 million m^3 (of which 3-4 million m^3 in Sweden alone) (Biedermann *et al.*, 2019; Tomás Hlásny *et al*., 2019; Öhrn *et al*., 2021).

Due to its timber value, Norway spruce has been planted extensively throughout Europe, including in areas outside its native range (Christiansen & Bakke, 1988). The total growing stock of Norway spruce is currently estimated to be 7.0 billion m^3, all of which is exposed to *I. typographus* (Tomáš Hlásny *et al*., 2021). Therefore, outbreaks in managed spruce plantations can significantly affect the stability of regional economies and markets and generate political conflicts (Morris *et al.*, 2017; Montagné-Huck & Brunette, 2018). The economic effect is caused by (*i*) decreasing timber prices in the short-term, due to oversupply resulting from salvage logging of large infested areas and (*ii*) increased timber prices in the long-term, due to the reduced availability of timber on the market (Tomáš Hlásny *et al*., 2021).

In unplanted natural forests, larger outbreaks typically have other types of consequences. For example, they can affect ecosystems and the services they supply (*e.g*. biogeochemical cycles, biodiversity) and result in major transformation of forest landscapes (Raffa *et al*., 2008; Thom & Seidl, 2016).

In the near future, bark beetle outbreaks are predicted to increase as a consequence of the high number of planted suitable host trees and changing climate conditions (Raffa *et al*., 2008; Bentz *et al*., 2019). Warm, dry weather conditions can accelerate the development of bark beetles, reduce tree defence, and facilitate expansion of bark beetles into new territories with low pressure from natural enemies (Seidl *et al*., 2008; Buotte *et al*., 2016; Seidl & Rammer, 2017).

Management of *I. typographus* typically involves reactive measures, *e.g*. silvicultural practices that most commonly consist of clearing windthrown timber or felling and removing infested trees (Wermelinger, 2004). However, since the rate of outbreaks is predicted to increase in coming years, new management strategies must be considered. One potential approach relies on preventive measures, through creating favourable habitat conditions for natural enemies of bark beetles.

Bark beetles are attacked by diverse groups of natural enemies, such as pathogens (fungi, bacteria), parasites (nematodes), parasitoids (wasps), and predators (clerid beetles, flies, mites and woodpeckers) (Wegensteiner *et al*., 2015). These natural enemies are known to significantly reduce bark beetle populations under natural conditions and can be included in biological control programmes to safely and sustainably regulate populations of bark beetle species (Kenis *et al*., 2019).

One important group of organisms acting as a natural enemy of bark beetles contains predatory larvae of the long-legged fly species *Medetera* Fisher von Waldheim 1819 (Diptera: Dolichopodidae) (De Leon, 1935; Hopping, 1947; Beaver, 1966; Fitzgerald & Nagel, 1972; Bickel, 1985). However, these species are understudied, which limits their potential application in pest management strategies. A thorough understanding of *Medetera* biology, sensory ecology and behaviour is a key to implementation of an integrated strategy for controlling tree-killing bark beetle species.

The main aim in this thesis was to identify odours used by predatory *Medetera* flies to detect Norway spruce trees infested with the Eurasian bark beetle (*I. typographus*). Odours were sampled and studied for their chemical identity, antennal activity and attractiveness for *Medetera* flies in forest conditions. A second aim was to describe the morphology of the peripheral olfactory system in the two most commonly found *Medetera* species in southern Sweden, and using DNA barcoding as a complement to morphological *Medetera* species identification. A final aim was to perform

a brief review of prey associations between 30 *Medetera* species and tree-killing bark beetle species. The results obtained were intended to extend existing knowledge on the ecology of *Medetera* species and contribute to the development of a monitoring bait that can be optimised for future implementation.

2. Research Background

2.1 Tree-killing bark beetles

There are approximately 6000 species of bark beetles distributed around the world. Most of these species breed in dead or dying trees, where they play an important role in the decomposition and nutrient cycling of wood (Raffa *et al*., 2008; Raffa *et al*., 2015). However, when present in high population densities, some of these species are also able to attack and kill healthy trees over large areas (Kausrud *et al*., 2012; Biedermann *et al*., 2019).

Bark beetle outbreaks occur during intermittent events. In endemic phases, beetle populations are controlled by tree resistance, weather, competitors and natural enemies (Raffa *et al*., 2008). However, storm disturbances, warm temperature and periods of low precipitation can increase the population density by reducing tree resistance and/or increasing the number of bark beetles (Kausrud *et al*., 2012; Marini *et al*., 2017; Kamińska *et al*., 2021). In such cases, bark beetles are capable of overcoming healthy, well-defended trees via mass attacks and starting an epidemic phase (Wallin & Raffa, 2004; Boone *et al*., 2011; Kausrud *et al*., 2012).

This chapter describes the life cycle of Eurasian *Ips typographus,* the importance of pheromone-mediated mass attacks and symbiotic microorganisms to overcome conifer chemical defence, common pest management strategies and biological pest control.

2.1.1 Eurasian bark beetle (*Ips typographus*)

A generalised diagram of the life cycle of *I. typographus* on Norway spruce trees is presented in Figure 1. During spring, overwintering adult bark beetles of this specie emerge from hibernation sites and search for suitable host trees.

Males are known to arrive at host trees before females and start tunnelling small nuptial chambers in the inner bark. At the same time, males release an aggregation pheromone made up of two components, (–)-*cis*-verbenol and 2-methyl-3-buten-2-ol, that attracts and directs a large number of conspecifics of both sexes to the same host tree leading to a mass attack (Lanne *et al*., 1989; Lindström *et al*., 1989; Blomquist *et al*., 2010). During an attack, the beetles infect trees with symbiotic microorganisms that eventually metabolise tree defence chemicals (Krokene, 2015).

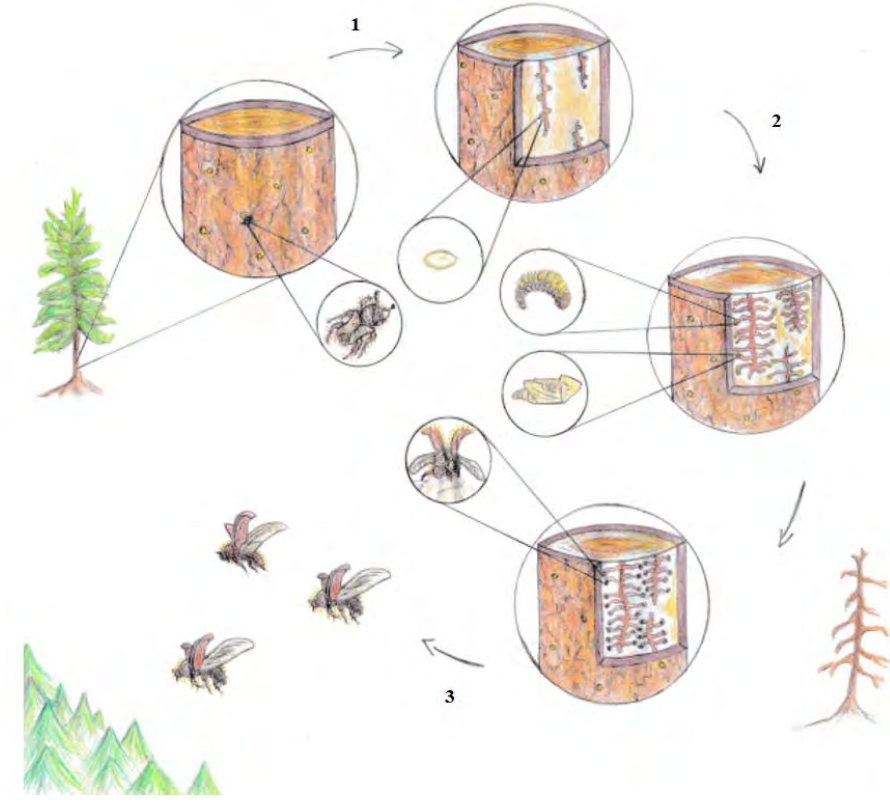

Figure 1: Generalised life cycle of Eurasian bark beetle (*Ips typographus*) on living Norway spruce trees, divided into three main phases. (1) Colonisation: adults construct galleries under the bark where they mate and lay eggs. (2) Brood development: eggs hatch, larvae grow and feed on the cambium and phloem. During feeding new tunnels are constructed away from the female galleries and at the end of each larval tunnel a pupal chamber is constructed, where the larvae pupates. (3) Dispersal: the new adults emerge and search for a new host tree.

Once tree defence is overwhelmed by the associated microorganisms, mating occurs in the nuptial chambers constructed by the males. After mating, females construct vertical maternal galleries for oviposition and the eggs are laid alternately along both sides of the maternal galleries (Mills, 1986). Few days later, the larvae eclose and feed on both cambium and phloem tissue.

During larval feeding new tunnels are constructed away from the female galleries. A pupal chamber is constructed at the end of each larval tunnel, and this is where the larvae pupate. After pupation, emerged adults either leave the tree and search for new host trees to initiate a new seasonal generation of beetles, or they overwinter as adults in the soil. When not fully developed, bark beetle larvae overwinter under the bark of infested trees (Wermelinger *et al*., 2012). However, winter has a significant impact on the survival of bark beetle larvae, *e.g*. about 50% of bark beetle larvae are estimated to die if the temperatures fall below -10 °C (Faccoli, 2002).

Development of a full generation usually takes about 6-10 weeks and depending on temperature and elevation, *I. typographus* can have one, two, or possibly three generations per year (Wermelinger, 2004). In Central Europe, there are usually two generations per year, while at high altitude (>1500 m a.s.l.) or further north, the populations are usually univoltine (one generation per year) (Wermelinger & Jakoby, 2022).

2.1.2 Attacks on living trees and the importance of symbiotic microorganisms

Trees have a sophisticated defence system to withstand attack by bark beetles, which includes the formation of necrotic lesions around the tree tissue infested with the beetle, and the production of high levels of phenolic and terpenic compounds (volatile mono- and sesquiterpenes or non-volatile diterpenes) that are toxic to different life stages of bark beetles (Martin *et al*., 2003; Keeling & Bohlmann, 2006). However, through pheromone-mediated mass attack and with the help of plant-pathogenic microbes, the beetles can overcome tree defences (Christiansen & Bakke, 1988; Kirisits, 2004; Krokene, 2015). For example, *I. typographus* is associated with ectosymbiotic, phytopathogenic blue-staining fungi of the genera *Endoconidiophora*, *Ophiostoma* and *Grosmannia*, which the beetles transfer to the host tree during colonisation (Paine *et al*., 1997; Kandasamy *et al*., 2016; Kandasamy *et al*., 2021). These symbiotic microorganisms are known

to invade healthy sapwood, disrupt water transport and overcome host tree defences (Horntvedt *et al*., 1983; Kirisits, 2004). Moreover, they can metabolise host defence compounds (Boone *et al*., 2013; Hammerbacher *et al*., 2013; Zhao *et al*., 2019). According to Kandasamy et al. (2023) the *I. typographus* symbiotic fungus *Grosmannia penicillata* increases conversion of the terpene-rich defensive resin in host tree bark into various oxygenated monoterpenes, *e.g*. bornyl acetate can be metabolised into camphor and α-pinene, while β-pinene can be metabolised into trans-4-thujanol and other oxygenated products.

2.1.3 Management strategies

Pest management practices currently aim at prevention of *I. typographus* mass attacks and keeping tree damage at a minimum. At present, sanitation felling and removal of infested trees from forests is regarded as one of the most effective management methods against *I. typographus* (Tomás Hlásny *et al*., 2019). Sanitation felling involves felling and removal of bark beetle infested standing trees and removal of windthrown timber that could be used by the bark beetles as a breeding substrate (Wichmann & Ravn, 2001; Wermelinger, 2004). However, the efficiency of these management activities depends on three critical elements: (*i*) early detection of newly infested trees; (*ii*) infested trees being removed before emergence of the new generation of bark beetles; and (*iii*) effective treatment to kill the brood before or after moving the logs to collection sites (Wermelinger, 2004). It is also important to acknowledge that sanitation felling can negatively affect habitats and lower the abundance of beneficial insects, and that removal of dead trees, especially after emergence of bark beetles, may still harm remaining natural enemies (Martikainen *et al*., 1999; Aukema *et al*., 2000).

Bark beetles are associated with several groups of natural enemies, *e.g*. woodpeckers, parasitoids, predators and pathogens (Wegensteiner *et al*., 2015). Natural enemies, such as parasitoids, predators and pathogens, can be used in biological programmes to safely and sustainably regulate populations of tree-killing bark beetle species. Biological control can be applied in three different approaches (*i*) *augmentative*: release of natural enemies that already exist in the affected area, to increase their density; (*ii*) *conservation*: manipulation of the local habitat to enhance reproduction, survival and efficacy of resident natural enemies already present in the affected areas; and (*iii*) *classical*: introduction of a new natural enemy into the habitat to control

the pest density (Klapwijk *et al*., 2016; MacQuarrie *et al*., 2016; Kenis *et al*., 2017; Hajek & Eilenberg, 2018; Stenberg *et al*., 2021). Although biological control has not been commercially established against *I. typographus*, programmes for the biological control of other tree-killing bark beetle species such as *Dendroctonus micans* Kugelann using the predatory beetle *Rhizophagus* grandis Gyllenhal (Coleoptera: Rhizophagidae), have been successfully carried out in France, United Kingdom and Turkey (Grégoire *et al*., 1992; Evans & Fielding, 1994). Biological control of *Ips grandicollis* Eichhoff in Southern Australia has also been attempted using different natural enemies (Waterhouse & Sands, 2001).

2.2 Long-legged flies of the genus *Medetera*

Long-legged fly species of the genus *Medetera* (Diptera: Dolichopodidae) are predators of a wide variety of arthropods. The adult flies are often found on vertical surfaces such as tree trunks, walls or rocks, where they feed on small soft-bodied prey such as Collembola, Psocoptera and small Diptera (Ulrich, 2004). Predatory larvae of some *Medetera* species that live under the bark of dead or dying trees are reported to prey on Scolytinae species that are known to attack and kill conifer and broadleaf trees (Bickel, 1985).

The following sub-sections provide a general overview of the life history of the different species preying on tree-killing bark beetles and their potential effect as biological control agents.

2.2.1 Species known to prey on bark beetles

So far, about 30 of the more than 300 species of the genus *Medetera* described worldwide have been found to be associated with tree-killing bark beetles (Yang, 2006; Negrobov & Naglis, 2016; **Paper I**). The number of species preying on tree-killing bark beetles seems to be higher in Central and Northern Europe than in North America (Negrobov & Naglis, 2016). Overall, larvae of different *Medetera* species have been associated with one or several tree-killing bark beetle species within the genera *Dendroctonus*, *Dryocoetes*, *Hylurgops*, *Hylastes*, *Ips*, *Orthotomicus*, *Polygraphus*, *Pityogenes*, *Pityophthorus*, *Pseudohylesinus*, *Scolytus*, *Taphrorychus,* and *Tomicu*s (Supplementary Table S1/**Paper I**).

Although the potential of these *Medetera* species as biological control agents of tree-killing bark beetles was noticed already in the early 1930s (if

not before) (De Leon, 1935), information regarding their biology, ecology and association with Scolytinae prey is still scarce. The main reason for this lack of information may be that *Medetera* flies are difficult to identify based on morphological characters. Most of the species are primarily identified based on the fine structure of male genital morphology, while there are almost no available morphological keys for female flies (Pollet *et al.*, 2011; Pollet et *al*., 2022).

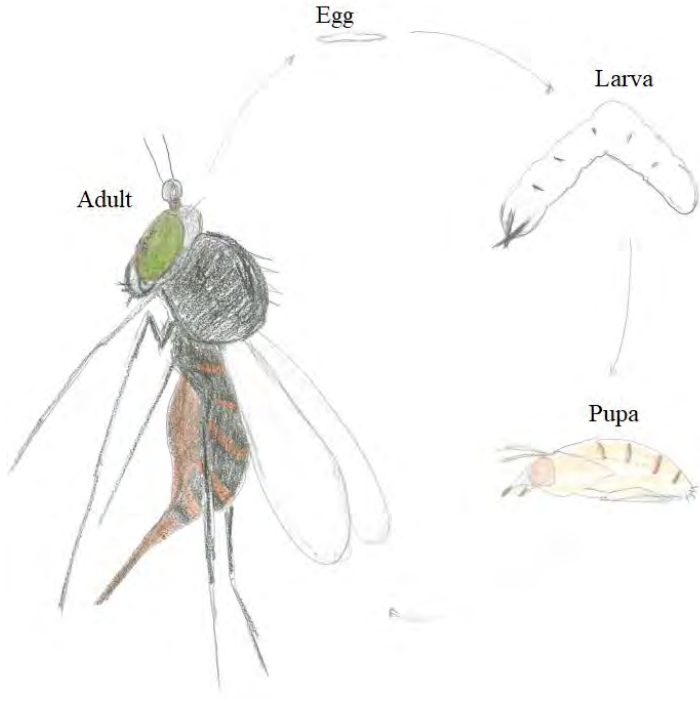

Figure 2: Life stages of *Medetera* species. In this genus, three to four different larval instars can be distinguished. The adult fly in the diagram is a *M. signaticornis* female.

The life stages of *Medetera* species are presented in Figure 2. *Medetera* species preying on tree-killing bark beetles are known to emerge from infested trees during spring, almost simultaneously with their Scolytinae prey (Beaver, 1966; Nicolai, 1995). After emergence, the flies move to other trees. To date, it is not clear whether subsequent mating occurs in the same tree where oviposition occurs, or at another site (Hopping, 1947; Bickel, 1985). In any case, gravid females are known to arrive at host trees shortly

after initiation of a bark beetle attack, and their presence and oviposition on individual trees can be continuously observed throughout the summer (Stephen & Dahlsten, 1976; Nicolai, 1995; Aukema & Raffa, 2004; Wermelinger, 2004). According to Beaver (1966), both *M. nitida* and *M. impriga* have a prolonged period of egg-laying on the same tree infested with bark beetles, where almost fully developed larvae can be found at the same time as newly hatched larvae.

On infested trees, females *Medetera* inspect the bark surface for an oviposition site by exposing the tip of the long ovipositor to the bark (Beaver, 1966). Oviposition occurs in bark crevices or under scales near the entrances to bark beetle galleries (Hopping, 1947; Fitzgerald & Nagel, 1972; Bickel, 1985), preferably at the lower part of the tree trunk (Wermelinger, 2002). One female lays between one to four eggs at a time, but can deposit more than 100 eggs during her life (Beaver, 1966). For example, *M. dendrobaena* females have been observed to produce up to 120 eggs (Dippel *et al*., 1997). The eggs hatch over a period of 10 days (Beaver, 1966) and the newly eclosed larvae move from the oviposition sites into the bark beetle larval galleries towards their prey (Nagel & Fitzgerald, 1975). Even when *Medetera* larvae are unable to penetrate unmined phloem, they can slowly move through prey galleries that are tightly packed with frass, with their movement seeming to be facilitated after the mined inner bark begins to dry out (Nagel & Fitzgerald, 1975).

Once inside the bark beetle galleries, *Medetera* larvae can prey on all developmental stages of bark beetles (eggs, larvae, pupae and/or newly emerged callow beetles that are still concealed in the galleries and pupal chambers) (Beaver, 1966; Bickel, 1985). When prey is found, *Medetera* larvae attack it with their tentorial rods and inject a venom. Once the prey is immobilised, the *Medetera* larvae rupture the prey's integument with their mandibular hooks and suck out the fluid within (Aukema & Raffa, 2004). Depending on the size of the prey, one *Medetera* larva can consume between five and 20 individuals during development. If the amount of prey is high, *Medetera* larvae kill more than they can feed on, while if food is scarce they can be cannibalistic (Beaver, 1966; Nicolai, 1995). The diet of *Medetera* larvae seems not to be restricted to Scolytinae, and they may also prey on Hymenoptera and on other Diptera species that live under tree bark (Beaver, 1966). However, information about this is very scarce.

Under laboratory conditions, four larval stages of *M. dendrobaena* can be distinguished (Nicolai, 1995). When the larvae reach the mature stage, but before pupation, they move to places where the adults can easily exit. According to Fitzgerald (1968), pre-pupal *M. aldrichii* larvae are photosensitive and usually follow the light entering the bark beetle galleries to locate potential exit sites. Once a site for pupation is selected, a cocoon is first constructed, in which the larva lies in a "U" shape for one week or longer before pupation. After the cocoon is formed, the pupation phase can last for about 18 to 21 days (Beaver, 1966; Nicolai, 1995).

Medetera species can have one or several generations depending on the length of life cycle of the particular species and weather conditions. According to Beaver (1966), in general the *Medetera* life cycle has a minimum length of about seven and a half weeks. At one site in that study, only one generation per year was found for *M. nitida,* since the adults were observed during a short period from mid-June until late August. At another site in the same area, two (possibly three) generations might occur for *M. impigra*, since adults were observed for a longer period (May until mid-October) (Beaver, 1966).

During winter, *Medetera* larvae stay under the bark of infested trees (Beaver, 1966; Weslien, 1992; Nicolai, 1995; Wermelinger *et al*., 2012). All instars of *M. impigra* larvae can overwinter in Scolytinae larval galleries, but the last instar (pupating) larvae become quiescent, while immature larvae continue to feed if the ambient temperature is sufficiently high (Beaver, 1966). However, winter can have a significant impact on the mortality of *Medetera* species (Hopping, 1947). According to Nicolai (1985), larvae of *M. dendrobaena* lose weight over the winter and by the spring only 20% of the larvae have a body mass >3.0 mg, the level which has been determined to be necessary for successful pupation when the temperature increases.

2.2.2 Prey location

How the different *Medetera* species locate bark beetle-attacked trees or how they locate their prey under the bark of an infested tree has been studied to a limited extent. As observed for other natural enemies of bark beetles, olfaction likely plays an important role for prey location and oviposition by *Medetera* species (Tømmerås, 1985; Goheen & Hansen, 1993; Pettersson *et al.*, 2000; Pettersson, 2001).

It has been shown that *Medetera* adult flies are attracted to the pheromone components emitted by their Scolytinae prey. For example, in North America *M. bistriata* is attracted to frontalin, a key pheromone component produced by bark beetles species of the genus *Dendroctonus*, such as *D. frontalis* and *D. brevicomis* (Vité & Pitman, 1969; Williamson, 1971). In Europe, *M. melancholica* and *M. setiventris* are reported to be attracted to the aggregation pheromone components produced by *I. typographus* (Hulcr *et al*., 2005; Hulcr *et al*., 2006). In addition to bark beetle pheromones, *Medetera* adult flies are able to detect tree-produced monoterpenes, *e.g.* α-pinene, β-pinene and limonene (Rudinsky *et al*., 1971; Hulcr *et al*., 2005, 2006). According to Fitzgerald (1962), α-pinene can stimulate oviposition in gravid *M. aldrichii* females. *Medetera* adult flies have also been shown to be capable of detecting logs infested with microorganisms that live in symbiosis with bark beetles (Boone *et al.,* 2008). For example, according to Boone *et al*. (2008), unidentified *Medetera* species are more attracted to logs colonised by fungi (*e.g*. *Ophiostoma ips*) or a bacterial strain (*Burkholderia* sp.) associated with bark beetle compared with uncolonised logs.

In addition to olfaction, visual cues such as colour, bark texture, form and contrast may be important in orientating and promoting the landing of *Medetera* adult flies on a suitable tree infested with bark beetles. According to Goyer *et al*. (2004), the number of *M. bistriata* attracted to *Ips*-infested logs is strongly affected by colour and season. In that study, it was observed that white logs caught 50-56% fewer flies than black logs and that fly orientation was dramatically affected by season. In spring, flies showed preference for vertical logs compared with horizontal logs, but this preference decreased during summer and reversed during autumn (Goyer *et al*., 2004).

2.2.3 Predation - Impact on bark beetles

The impact of predators on tree-killing bark beetles depends *e.g*. on the level of synchrony between predator and prey life cycles, predator and prey abundance and predator consumption rates. *Medetera* species are among the most abundant predators found on infested bark beetle logs (Weslien, 1992; Wermelinger, 2002). Under laboratory conditions, the number of bark beetles killed by *Medetera* larvae has been found to be density-dependent, *i.e.* the higher the density of bark beetle larvae per log, the greater the number

of bark beetle larvae killed by each *Medetera* larva (Beaver, 1966; Nicolai, 1995).

Several studies have examined mortality rates of tree-killing bark beetles caused by *Medetera*. Under laboratory conditions, it has been shown that *Medetera* larvae can destroy up to 32% of larvae of the bark beetle *Hylurgops palliatus* (Nuorteva, 1956). Similarly, larvae of *M. nitida* have been shown to account for about 25% of the mortality of *Scolytus multistriatus* attacking broadleaf trees (elm) (Beaver, 1966). In another study, around 100 *M. dendrobaena* emerged per m^2 of spruce bark infested with *Pityogenenes chalcographus*, and were estimated to cause about 45% mortality of this beetle species under the bark (Nicolai, 1995). Under field conditions, assuming that one *Medetera* larva eats about five *I. typographus* larvae and considering the observed density range of 20-90 larvae per m^2 of infested logs, it has been estimated that around 100-500 *I. typographus* offspring per m^2 can be consumed by *Medetera* larval offspring (Weslien & Regnander, 1992). Similarly, in a study in the Sernftal valley near Schwanden in Switzerland during 1994 and 1995, predators of the genus *Medetera* were observed to be the second most common group of natural enemies emerging from *I. typographus*-infested logs (Wermelinger, 2002). Due to their high densities and voracity, together with parasitoid wasp species of the *Roptrocerus* genus they were estimated to account for more than 80% of bark beetle mortality in that study (Wermelinger, 2002).

3. Aim and objectives

The overall aim of this thesis was to provide new knowledge on the ecology of selected species within the genus *Medetera* that prey on tree-killing bark beetles, especially regarding their ability to locate bark beetle infested trees.

In **Papers I** and **II** the identity and morphology of species within the genus *Medetera* that function as bark beetle predators were investigated. Specific objectives of these two studies were to:

- Identify different *Medetera* species associated with the Eurasian spruce bark beetle *I. typographus* in Swedish forest.
- Confirm the congruence between morphological and molecular identifications using DNA barcoding.
- Describe and compare the morphology of the olfactory organs and sensilla equipment of the two most abundant *Medetera* species at the study sites.

Papers III and **IV** investigated the response of *Medetera* species to odours from Norway spruce trees infested with the Eurasian spruce bark beetle *I. typographus*. Specific objectives of these two studies were to:

- Identify odours emitted from Norway spruce trees infested with *I. typographus* that are antennally active on *Medetera signaticornis*.
- Test the behavioural response of *Medetera* species to synthetic blends that contain different combinations of antennally active odours.

4. Methodology

This chapter briefly presents the methods used in the different studies described in Papers I-IV. For full details of all methods, see the respective papers.

4.1 Morphology vs molecular analyses

4.1.1 Morphological identification

Morphological identification is the conventional method used to identify organisms, on the basis of morphological features that characterise the species. Identification keys that summarise and compare anatomical features distinguishing different species are widely used as a tool to identify species. In **Papers I-IV,** adult fly specimens of the genus *Medetera* were morphologically identified to species level using the morphological key developed by Negrobov and Naglis (2016), in combination with diagrams in publications by Negrobov and Stackelberg (1972, 1974a, 1974b) and Negrobov (1977). All specimens were morphologically identified by Marc Pollet, an expert with almost 40 years of experience in identifying flies within the Dolichopodidae family. When possible and needed, representative identified specimens (morphotypes) were compared with specimens from his reference insect collection.

The main morphological characters used to separate *Medetera* species in this thesis were: thoracic chaetotaxy (*e.g*. relative size of dorsocentral bristles, number of scutellar bristles), colour of scape and pedicel (*e.g*. yellow in members of the *Medetera signaticornis-pinicola* species group, dark in most other *Medetera* species), shape and size of the postpedicel (*e.g*.

elongated in *M. ambigua*, subtriangular in *M. pinicola*, quadrate in *M. signaticornis*), colour of the halter (*e.g.* dark in *M. ambigua* and *M. signaticornis*, pale in *M. pinicola*), chaetotaxy of the femora (*e.g.* strong ventral bristles present or absent) and shape of vein M1 (straight or distinctly curved). However, the most decisive characteristics were the shape of the genital appendages in male flies.

4.1.2 DNA barcoding

DNA barcoding has been firmly established as a genome-based method for taxon identification of all living things (Ankola *et al.*, 2021). Identification of insects and other animals involves a short sequence (~658 bp) of mitochondrial cytochrome oxidase subunit I (COI) that enables fast and reliable taxon identification to species level across any life stage (Hebert *et al.*, 2003a; Hebert *et al*., 2003b). In **Paper I**, COI sequences were extracted from adult fly specimens of the genus *Medetera* collected from Norway spruce trees in southern Sweden infested with the bark beetle *I. typographus*. The COI sequences were used to study congruence between morphological and genetic identifications of the species found at field sites.

4.1.3 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is a technique that produces high-resolution images of a sample by scanning the surface with a focused beam of electrons (Cheney, 2007). In **Paper II**, SEM was used to examine and compare the morphology of the antenna, maxillary palps and sensilla equipment of the two most abundant species of the genus *Medetera* found at the field sites (*M. signaticornis* and *M. infumata*).

4.2 Odour collection and analyses

Odours emitted from plants are a mixture of volatile compounds that can be collected from the air surrounding the plant using different headspace sampling techniques (Tholl *et al*., 2006). In nature plants are associated with other organisms such as fungi or arthropods that influence the emission of plant odours and also emit their own volatiles (Farré-Armengol, 2016; Karamonoli *et al*., 2020). In **Paper III**, volatile compounds were collected at several time-points throughout different stages of *I. typographus* attacks

from cut and standing Norway spruce (*Picea abies*) trees, and also from non-infested trees. First, to capture compounds emitted from the bark surface and reduce the entry of contaminants, around ~9 dm^2 of the bark surface was enclosed using an aluminium grid and a polyester bag. The volatiles released inside the enclosed area were collected on an adsorbent material (Porapak Q, mesh 50/80) using an air pull system through the adsorbent columns. The volatile compounds were then desorbed using an organic solvent, pentane, and the solution was concentrated. Combined gas chromatography and mass spectrometry (GC-MS) was used to identify and quantify the collected volatile compounds. In the GC-MS technique the volatile compounds in a sample are separated by their affinity to the stationary phase of the GC column, and identified by their mass spectral matches (MS) in comparison to standard or library spectra, and by their relative retention time matches (*i.e*. Kovats Indices) in comparison to known standards, such as PheroBase®, ChemSpider®, and PubChem®.

4.3 Electroantennographic experiments

Antennae and maxillary palps are the two main olfactory organs of adult insects. These olfactory organs have hair-like structures called sensilla that detects stimuli in the environment (Schneider, 1964; Hansson & Stensmyr, 2011). Some types of sensilla have small wall pores where odours can diffuse and bind with specific olfactory receptors present in the dendritic membrane of olfactory sensory neurons. When an odorant molecule binds to an olfactory receptor, a chain-reaction is triggered resulting in activation of ion channels located in the dendritic membrane. This activation generates an electrical impulse that can be detected by electroantennography (Breer, 1997; Krieger & Breer, 1999).

In **Paper III**, combined gas chromatography and electroantennographic detection (GC-EAD) was used to screen for compounds collected from *I. typographus* infested spruce trees, that could elicit neural activity in the antennae of both males and females *M. signaticornis*. Compounds were categorised as biologically active if they elicited a reproducible response in fly antennae. Biologically active compounds were identified by their Kovats Indices, in combination with GC-MS analyses. Some of these biologically active compounds were then used to prepare synthetic baits for field assays.

4.4 Field trapping

Field trapping experiments were used to investigate the response of insects to synthetic compounds and to assess laboratory findings under more natural conditions. In **Paper III,** a field trapping experiment was performed to investigate whether *M. signaticornis* adults were effectively attracted by synthetic blends containing 18 compounds categorised as active in GC-EAD and two additional compounds, 2-methyl-3-buten-2-ol and ipsdienol, previously reported to be involved in attraction of other *Medetera* species (Hulcr *et al*., 2005; Hulcr *et al*., 2006). For this trapping experiment two different quantitative compositions of the synthetic chemicals were tested: (*i*) a 1:1 mix in which GC-EAD active and additional compounds were presented in equal proportions, and (*ii*) a natural mimic in which GC-EAD active and additional compounds were presented according to the amounts released from a 50 dm^2 area of a living infested Norway spruce tree at the beginning of a bark beetle *I. typographus* attack.

In **Paper IV**, the analyses were continued by testing the attraction of *Medetera* species, including *M. signaticornis* and *I. typographus,* to different combinations of the compounds previously tested in **Paper III** and two additional isomers (of previously tested compounds). In **Paper IV**, the compounds were divided in five groups (A, B, C, D and E) according to their primary biological origin. Two different subtractive combinations of these groups were tested in a Latin square and partial factorial experimental design. Compounds were prepared in heptane and released according to the calculated amounts released from a 1000 dm^2 area (*i.e*. a 15 m high tree trunk ~30 cm breast height diameter (BHD)) of a living infested Norway spruce tree at the beginning of a bark beetle (*I. typographus*) attack.

5. Summary of results and discussion

5.1 Characterising the identity and olfactory morphology of collected predatory *Medetera* species (Papers I and II)

Morphological identification of *Medetera* species is challenging. In **Paper I**, DNA barcodes were used to check the congruence between morphological and genetic identifications. Using this method, two cases of morphological mismatches were detected and it was possible to identify species identity for four out of nine specimens that could only be identified to genus level morphologically. These results indicate that DNA barcoding can be applied as a reliable tool to assess and speed up species identification of *Medetera* specimens that have similar or rare morphological features, or are damaged. DNA barcoding has already been successfully used for the identification of species from other insect groups that have rare morphological features or parts of insect bodies (Cocuzza *et al*., 2015; Powell *et al.*, 2019; Behrens-Chapuis *et al*., 2021). Barcoding can also be applied for the identification of the different developmental stages (adults, larvae and pupae) (Meiklejohn *et al*., 2013; Ståhls *et al*., 2009) and can be a useful tool for ecological studies of species community composition, genetic variation and gene flow (Bras *et al.*, 2019; Lopez-Vaamonde *et al.*, 2021, Pérez-Delgado *et al*., 2022). In-depth ecological knowledge about natural enemies is required to identify and select species for biocontrol applications of *I. typographus* or other tree-killing bark beetle species

A review of published data on the range of larval prey for *Medetera* species and their geographical distribution revealed that at least 30 different *Medetera* species are associated with tree-killing bark beetles (**Paper I**).

These include *M. abstrusa*, *M. fumida*, *M. pseudoapicalis*, *M. prjachinae,* and *M. zinovjevi*, which have only been reported in galleries of *I. typographus* attacking *Picea abies* in northern European forests (Hedgren & Schroeder, 2004). Others, such as *M. adjaniae*, *M. aldrichii*, *M. dichrocera*, *M. excellens*, *M. fascinate*, *M. melancholica*, *M. penicillata*, *M. pinicola*, *M. setiventris*, *M. signaticornis,* and *M. striata*, have been reported in galleries of bark beetle species of more than three different genera. Some of these generalist species have an Holarctic distribution (Bickel, 1985; Negrobov & Naglis 2016), and have been found preying on *Ips, Pityogenus*, *Hylurgops*, *Dendroctonus, Polygraphus* and *Dryocoetes* (Bickel, 1985; Wermelinger, 2002; Hedgren & Schroeder, 2004; **Paper I**). The results presented in **Paper I** comprise new knowledge about practical identification and systematics that may facilitate future research on the ecology and functional relevance of different *Medetera* species.

Comparative analysis of the morphology of the olfactory organs and sensillar equipment of *M. signaticornis* and *M. infumata,* the two most common species found at the study sites in **Paper I**, showed similar general morphology of the antennae and maxillary palps, in relation to the shape, size and position (**Paper II**/Sousa et al. 2023a). However, there were differences between the species in terms of the subtypes and densities of antennal sensilla (*trichodea* and *basiconica*) (**Paper II**/Sousa et al. 2023a). It is known that types and abundance of sensilla often vary between insect species, reflecting species and sex specific adaptations that facilitate *e.g*. the location of prey, habitat or mates (Hansson & Stensmyr, 2011). Functional analysis of *sensilla basiconica* in other insect species has demonstrated that this sensillum type usually responds to a wide variety of odorants, including those involved in the detection of food and oviposition sites (De Bruyne *et al*., 1999; De Bruyne *et al*., 2001; Elmore *et al*., 2003), while the *s. trichodea* type has been shown to respond to chemical signals that facilitate *e.g*. foraging, oviposition and mate seeking (De Bruyne *et al*., 2001; Hill *et al*., 2009; Qiu *et al*., 2006). Based on this information, the differences observed in **Paper II** between *M. signaticornis* and *M. infumata* in terms of the subtypes and densities of *s. basiconica* and *trichodea* may suggest that the species respond to different environmental cues or process the same cues in different ways.

5.2 Odours from *Ips typographus* infested logs that are antennally active on *Medetera signaticornis* (Paper III)

Odours emitted from Norway spruce trees infested with the bark beetle *I. typographus* consist of a complex mixture of compounds. Some of these compounds are produced by the host tree as a defensive response to bark beetle attack. Others (*e.g*. aggregation pheromone) are produced by bark beetles living under the bark, and by bark beetle symbiotic microorganisms that have been introduced into the tree phloem during beetle colonisation (Birgersson *et al*., 1984; Duan *et al*., 2020; Kandasamy *et al*., 2023; **Paper III/**Sousa et al. 2023b) (Figure 3). In agreement with other studies, the qualitative and quantitative composition of these odours also varied throughout the different stages of *I. typographus* attacks (Birgersson *et al*., 1984; Pettersson & Boland, 2003) (**Paper III/**Sousa et al. 2023b).

The GC-EAD analyses in **Paper III** revealed that both *M. signaticornis* males and females were able to detect several compounds in a headspace sample collected from early infested bark beetle trees (Figure 4). The flies responded to (–)-*cis*-verbenol, (+)-*trans*-verbenol and myrtenol, compounds produced by *I. typographus* bark beetles (Birgersson *et al*., 1984; Birgersson & Bergström, 1989). The flies also responded to verbenene, isoterpinolene, α-pinene oxide, camphor, pinocamphone, terpinen-4-ol, myrtenal, borneol, α-terpineol, verbenone and geranyl acetone, compounds that are primarily produced by microorganisms associated with *I. typographus* (Leufvén *et al*., 1984, 1988; Kandasamy *et al*., 2016; Kandasamy *et al*., 2021). In addition, they responded to α-pinene, α-fenchene, β-pinene, camphene, 3-carene, limonene, γ-terpinene, and terpinolene, compounds produced by spruce trees (Duan *et al*., 2020; Netherer *et al*., 2021).

The compounds released by the host tree and bark beetles are released in greater amounts during the early stages of a bark beetle attack, while the oxygenated monoterpenes (*i.e*. camphor, pinocamphone) primarily produced by bark beetle symbiotic microorganisms are released in higher amounts during late stages of the attack (Pettersson & Boland, 2003; **Paper III**/Sousa *et al*., 2023b). Predatory *Medetera* species have been observed to arrive at freshly attacked trees almost simultaneously with bark beetles, possibly being attracted by the bark beetle aggregation pheromone. However, the adult flies have also been found on infested trees during late

Figure 3: Abundance (square root of mean amount released per surface area and unit time ($ng(dm^2s)^{-1}$)) of compounds detected in headspace samples collected from cut Norway spruce trees (*Picea abies*) infested with spruce bark beetles (*Ips typographus*), standing infested trees and non-infested trees. Odours were sampled from trees at two forest sites (S1 and S2) in up to seven sequential collections (C1 to C7) from 10 May (for S1) or 25 May (for S2) to 25 July 2018. The date of collection C1 differed slightly between sites, as bark beetles were active earlier at S1 than S2. However, the C1-C6 samples from attacked trees at site 1 represent similar stages of attack as samples C1-C6 collected at site 2. The compounds are listed in order of their gas chromatograph (GC) retention times. Asterisks (*) indicate significant differences in abundance of compounds (Multipatt, *$P \leq 0.05$; **$P < 0.01$; $P < 0.001$); n=no. of trees used at each site per treatment. Compounds in bold have been effectively identified with synthetic standards.

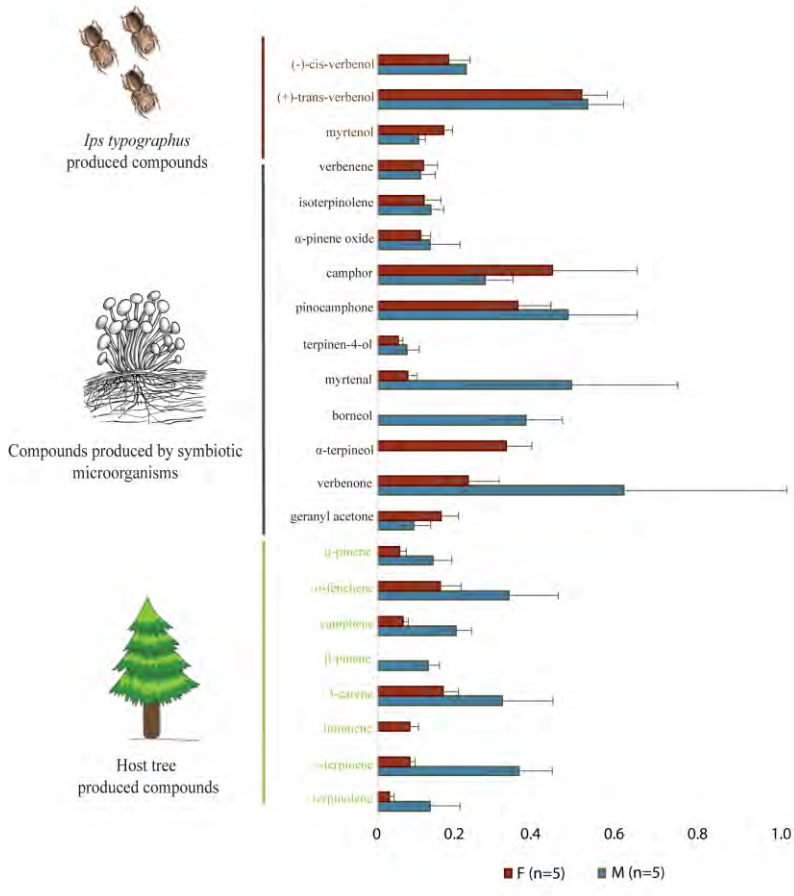

Figure 4: Combined gas chromatograohy and electroantennographic detection (GC-EAD) responses of female (F) and male (M) *Medetera signaticornis* fly antennae stimulated with odours collected from early infested Norway spruce tree (*Picea abies*). Mean response (mV ±SE) to the active compounds is organised according to their source. Compounds in brown font are produced by the bark beetle *Ips typographus*, compounds in black font are produced by *I. typographus* associated microorganisms and compounds in green font are produced by the tree.

stages of bark beetle attacks, after emission of bark beetle pheromone has ceased (Lawson et al., 1996). The results obtained in **Paper III** suggest that *M. signaticornis* may use multiple semiochemicals to detect bark beetle infested Norway spruce trees throughout infestation. This multitrophic interaction (between spruce tree, beetles, microorganisms and flies) needs to be considered in future studies on *Medetera* attraction behaviour.

5.3 Trapping of *Ips typographus* and predatory *Medetera* using synthetic blends of compounds emitted by infested logs (Papers III and IV)

The field bioassays using synthetic blends comprising 18 compounds categorised as active in GC-EAD and two additional compounds (2-methyl-3-buten-2-ol and ipsdienol) known to attract other *Medetera* species (Hulcr *et al*., 2005, Hulcr *et al.*, 2006) confirmed that both males and females of *M. signaticornis* adult flies can be effectively trapped (**Paper III/** Sousa et al. 2023a).

In **Paper IV**, the attraction of *Medetera* species and *I. typographus* to different combinations of the compounds previously tested in **Paper III** and two additional isomers ((+)-terpinen-4-ol and (+)-borneol) was assessed using two different subtractive combinations. The results from the first subtractive bioassay in **Paper IV** showed that adult *Medetera* specimens were collected in higher numbers from synthetic blends that combined bark beetle produced compounds, *i.e.* 2-methyl-3-buten-2-ol, (–)-*cis*-verbenol, (+)-*trans*-verbenol, (–)-myrtenol and (±)-ipsdienol, host tree produced compounds *i.e.* (+)-limonene, (–)-limonene, α-terpinene and γ-terpinene, and compounds produced by bark beetle symbiotic microorganisms *i.e*. (±)-camphor, (–)-terpinen-4-ol, (+)-terpinen-4-ol, (–)-borneol and (+)-borneol (Figure 5).

Based on these results, new synthetic blends were prepared for the second subtractive bioassay in **Paper IV**, the results of which suggested that of the five compounds produced by the bark beetles, (–)-*cis*-verbenol seemed to be important in attracting *Medetera* flies (Figure 6). Synthetic blends without (–)-*cis*-verbenol seemed to lose their attractive effect. It remains unclear how *Medetera* flies locates bark beetle infested trees when pheromone production ends. One possible explanation is that some GC-EAD active compounds not included in the combinations tested in **Paper IV** (*e.g*. pinocamphone, α-pinene oxide, isoterpinolene) might be of behavioural relevance.

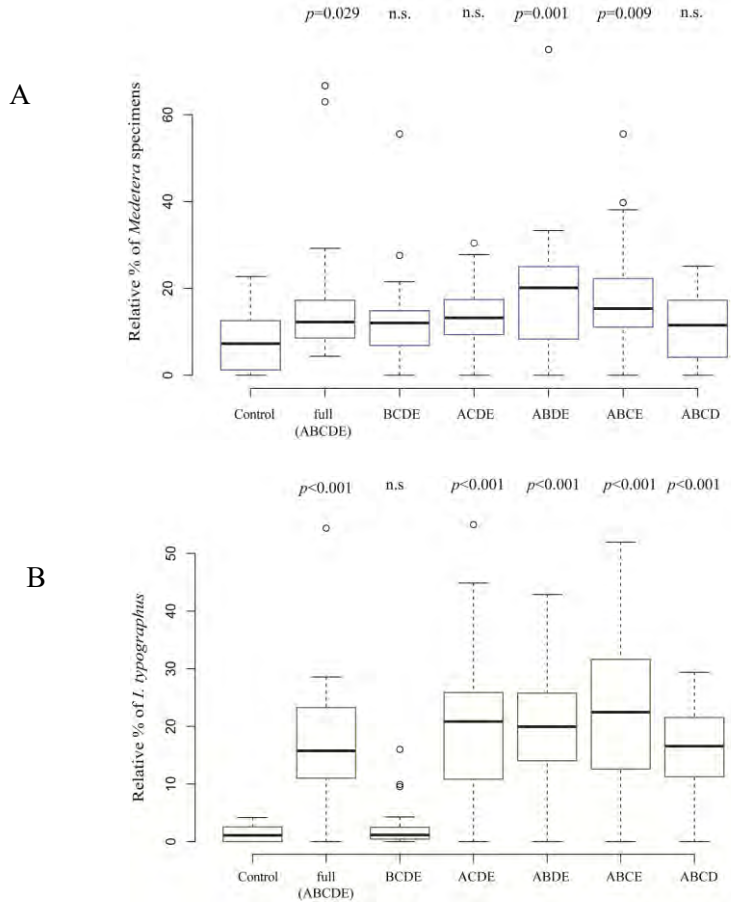

Figure 5: Boxplots showing the relative percentage (%) of trapped (A) *Medetera* flies and (B) *Ips typographus* beetles for the seven blends (six different synthetic blends, one control) used as baits in sticky traps during a 48-h trapping experiment conducted in seven test rounds at four different locations. Synthetic compounds emitted by bark beetle-infested Norway spruce (*Picea abies*) were divided into five different groups (A-E), where group A contained the bark beetle compounds 2-methyl-3-buten-2-ol, (–)-*cis*-verbenol, (+)-*trans*-verbenol, (–)-myrtenol, and (±)-ipsdienol; group B the microbial compounds (±)-camphor, (–)-terpinen-4-ol, (+)-terpinen-4-ol, (–)-borneol, and (+)-borneol; group C the microbial compounds (–)-myrtenal, (–)-verbenone, α-terpineol, geranyl, and acetone; group D the spruce tree compounds (±)-α-pinene, (–)-β-pinene, camphene, and terpinolene; and group E the spruce tree compounds α-terpinene, γ-terpinene, (–)-limonene, and (+)-limonene. A full blend (ABCDE) with all compounds and five blends with only four of the groups were tested in a subtractive test design. The control contained only the solvent heptane. The relative percentage of trapped *Medetera* and *I. typographus*, respectively was calculated for the individual treatments relative to the overall number of trapped specimens at the same time-point. P-values were calculated by pairwise comparisons between control and synthetic blends using the post-hoc *Dunnett's* test following two-way analysis of variance ($F=3.1$; $P<0.05$). Boxplots show the distribution of relative percentages between the lower, medians and upper quartiles, lines outside the boxplots (whiskers) indicate the variation outside the upper and lower quartiles. Open circles outside boxplots indicate outlier values that differed significantly from the rest of the dataset. n.s. = non-significant.

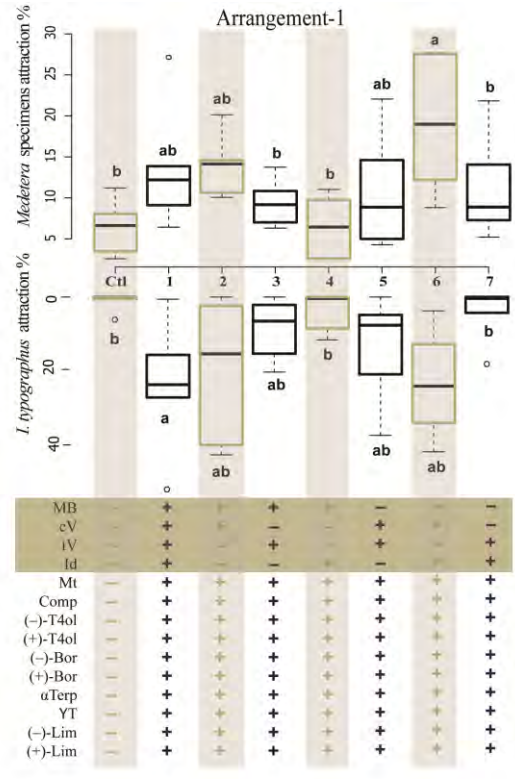

Figure 6: Boxplot showing the relative percentage (%) of trapped *Medetera* flies or *Ips typographus* beetles for different synthetic blends and solvent controls tested in a partial factorial design (2^{4-1}). The composition of the different blends is denoted by presence or absence of the individual compounds (bold + or -). Percentage of trapped insects was calculated from the original count obtained in each treatment divided by the sum of counts obtained in all treatments tested at the same time point, multiplied by 100. A general linear model (GML) followed by analysis of variance (ANOVA) was used to identify significant differences between the relative percentage of insects trapped by each synthetic blend. Arrangement-1 tested the main effects of bark beetle-produced compounds such as 2-methyl-3-buten-2-ol (MB), (–)-*cis*-verbenol (cV), (+)-*trans*-verbenol (tV), and (±)-ipsdienol (Id). Compounds such as (–)-myrtenol (Mt); (±)-camphor (Comp), (–) and (+)-terpinen-4-ol ((T4ol); (–) and (+)-borneol (Bor), α-terpinene (αTerp), γ-terpinene (γT), (–) and (+)-limonene (Lim) were added to all synthetic blends tested. The solvent heptane was used as a control (Ctl). The boxplot shows the distribution of the relative percentages between the lower, median and upper quartiles, lines outside the boxplots (whiskers) indicate the variation outside the upper and lower quartiles. The open circles outside the boxplot correspond to outlier values that significantly differ from the rest of the dataset. Different small letters (a,b) indicate significant differences ($p \leq 0.05$) between treatments according to *post-hoc* Tukey test.

At least 16 different *Medetera* species, morphologically identified, were found on the sticky traps baited with the synthetic blends (**Paper IV**). The most common were *M. signaticornis* (n=422), *M. infumata* (n=152), *M. prjachinae* (n=137), *M. setiventris* (n=78), *M. excellens* (n=33) and *M. ambigua* (n=29) (Figure 7). All these species have previously been reported on *I. typographus* infested Norway spruce trees (Hedgren & Schroeder, 2004; Wermelinger et al., 2012; **Paper II**/Sousa et al., 2023a). For all species except *M. infumata*, the number of females trapped was higher than the number of males. Thus, compounds released from Norway spruce trees infested with *I. typographus* most likely help *Medetera* females to find suitable oviposition sites, but may also help males and females to find sites for meeting and mating (Hopping, 1947).

In additional analyses, to understand more about the prey-predator interaction, the response of *I. typographus* to the synthetic blends was investigated during the same field trapping experiments (**Paper IV**). High numbers of trapped *I. typographus* were found in all traps baited with synthetic blends that contained either both components of bark beetle aggregation pheromone or just (–)-*cis*-verbenol (Figure 6). The response of *I. typographus* to components of aggregation pheromone is already well studied (Schlyter *et al*., 1987a; Schlyter *et al*., 1989). It has been shown that (–)-*cis*-verbenol acts as a long-range attractant, directing bark beetles to newly infested host trees, while 2-methyl-3-buten-2-ol acts as a short-range attractant directing the bark beetles to land on the infested host tree (Schlyter *et al*., 1987b).

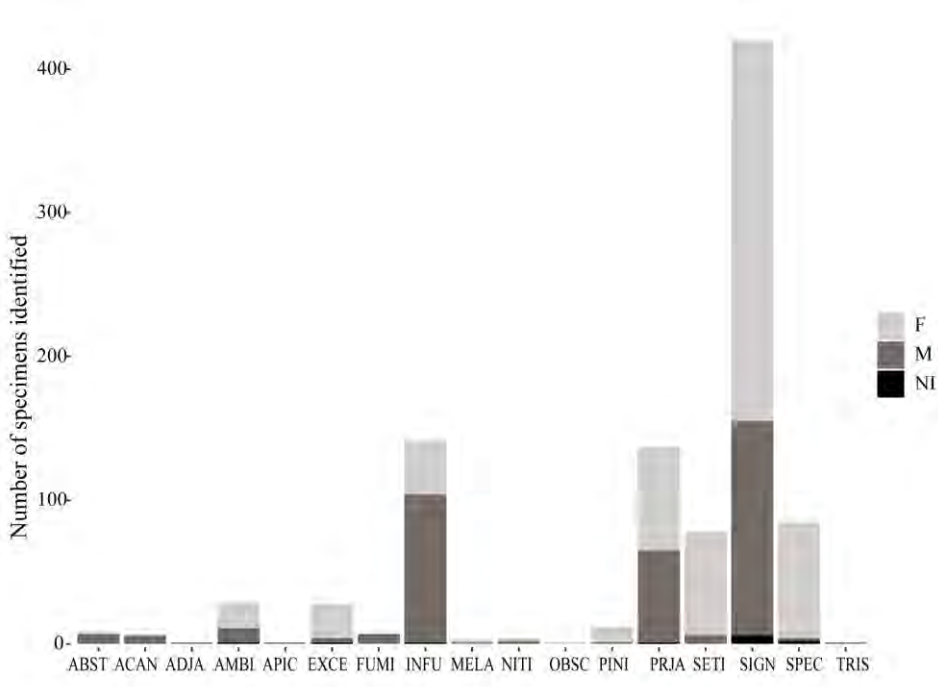

Figure 7: Total number of identified specimens (males and females) of different *Medetera* species collected at four different sites during 48 h periods (June 8-10; June 17-19; July 20-22) with sticky traps baited with six synthetic blends of compounds emitted by bark beetle-infested Norway spruce (*Picea abies*). Species names are abbreviated as follows: ABST = *Medetera abstrusa* Thuneberg, 1955; ACAN= *Medetera acanthura* Negrobov & Thuneberg, 1970; ADJA = *Medetera adjaniae* Gosseries, 1988; AMBI = *Medetera ambigua* Zetterstedt, 1843; APIC = *Medetera apicalis* Zetterstedt, 1843; EXCE = *Medetera excellens* Frey, 1909; FUMI = *Medetera fumida* Negrobov, 1967; INFU = *Medetera infumata* Loew, 1857; MELA = *Medetera melancholica* Lundbeck, 1912; NITI = *Medetera nitida* Macquart, 1834; OBST = *Medetera obscura* Zetterstedt, 1838; PINI = *Medetera pinicola* Kowarz, 1877; PRJA= *Medetera prjachinae* Negrobov, 1974; SETI = *Medetera setiventris* Thuneberg, 1955; SIGN = *Medetera signaticornis* Loew, 1857; TRIS = *Medetera tristis* Zetterstedt, 1838. F: females, M: males, NI: sex unknown SPEC: *Medetera* spp. identification was attempted, but not successful (mostly females).

6. Concluding remarks and perspectives

Species of the genus *Medetera* are reported to be natural enemies of tree-killing bark beetles. Understanding their host preference, the odorants they use to detect bark beetle-infested trees and how they interact with their prey is crucial for future planning and decision-making in the context of forest management and biological control.

In this thesis, approaches from multiple disciplines were combined and diverse aspects of the biology of predatory species within the genus *Medetera* were explored, including their morphology, prey associations and prey detection. However, some knowledge gaps remain.

First, there is a need for a more efficient identification method that can be applied on all life stages of *Medetera*. In this thesis, use of DNA barcoding as an identification tool is proposed, but a library of reference COI sequences for all *Medetera* species must be completed before this method can be effectively applied.

Second, associations of larvae of the different *Medetera* species with bark beetles and other insects is unclear. This thesis summarises information about bark beetle logs on which the larvae of the different *Medetera* species have been found to feed, or from which they emerge. However, published information seems to be limited to a few tree-killing bark beetle species. In addition, the impact of *Medetera* larvae on other beneficial insect species *e.g.* known to live under the bark of infested trees, has not been studied. A detailed understanding of the *Medetera* larval diet range is vital before using this organism for biological control of bark beetles. Further studies are necessary to identify the interactions between *Medetera* and bark beetles and/or between *Medetera* and other insects.

Third, the nature of odorants used by *Medetera* species to detect bark beetle-infested trees needs to be clarified. This thesis showed that *M. signaticornis* detects compounds produced by *I. typographus* bark beetles, bark beetle associated microorganisms and host trees. Based on these results, detection of compounds of different origins might facilitate the location of

infested trees throughout a bark beetle attack, especially at late stages when production of aggregation pheromone has ceased. However, it is unknown whether this finding also applies to other predatory *Medetera* species.

Last, a functional attractant to monitor *Medetera* is important to determine its presence or abundance before applying further management actions that might harm these natural enemies of bark beetle pests. The work in this thesis represents a significant step towards the development of a simple functional bait that can be used to monitor *M. signaticornis* and perhaps other *Medetera* species. However, the bait still needs to be optimised before practical implementation.

References

Ankola, K., Mahadevegowda, L., Melichar, T. and Boregowda, M.H., (2021). DNA barcoding: nucleotide signature for identification and authentication of livestock. *Advances in Animal Genomics*, 299-308.

Aukema, B.H. and Raffa, K.F., (2004). Behavior of adult and larval *Platysoma cylindrica* (Coleoptera: Histeridae) and larval *Medetera bistriata* (Diptera: Dolichopodidae) during subcortical predation of *Ips pini* (Coleoptera: Scolytidae). *Journal of Insect Behavior,* 17 (1), 115-28.

Aukema, B.H., Dahlsten, D.L., and Raffa, K.F., (2000). Exploiting behavioral disparities among predators and prey to selectively remove pests: maximizing the ratio of bark beetles to predators removed during semiochemically based trap-out. *Environmental Entomology,* 29 (3), 651-60.

Beaver, R.A., (1966). The biology and immature stages of two species of *Medetera* (Diptera: Dolichopodidae) associated with the bark beetle *Scolytus scolytus* (F.). *Physiological Entomology,* 41 (10-12), 145-54.

Behrens-Chapuis, S., Herder, F., and Geiger, M.F., (2021). Adding DNA barcoding to stream monitoring protocols–What's the additional value and congruence between morphological and molecular identification approaches. *PLoS One,* 16 (1), e0244598.

Bentz, B.J., Jönsson, A.M., Schroeder, M., Weed, A., Wilcke, R.A.I. and Larsson, K., (2019). *Ips typographus* and *Dendroctonus ponderosae* models project thermal suitability for intra-and inter-continental establishment in a changing climate. *Frontiers in Forests and Global Change,* 2, 1.

Bickel, D.J., (1985). A revision of the nearctic *Medetera* (Diptera: Dolichopodidae). United States Department of Agriculture, Agricultural Research Service.

Biedermann, P.H., Müller, J., Grégoire, J.C., Gruppe, A., Hagge, J., Hammerbacher, A., Hofstetter, R.W., Kandasamy, D., Kolarik, M., Kostovcik, M. and Krokene, P., (2019). Bark beetle population dynamics in the Anthropocene: challenges and solutions. *Trends in ecology & evolution,* 34 (10), 914-24.

Birgersson, G. and Bergström, G.,(1989). Volatiles released from individual spruce bark beetle entrance holes quantitative variations during the first week of attack. *Journal of Chemical Ecology,* 15 (10), 2465-83.

Birgersson, G., Schlyter, F., Löfqvist, J. and Bergström, G., (1984). Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases. *Journal of Chemical Ecology,* 10 (7), 1029-55.

Blomquist, G.J., Figueroa-Teran, R., Aw, M., Song, M., Gorzalski, A., Abbott, N.L., Chang, E. and Tittiger, C., (2010). Pheromone production in bark beetles. *Insect biochemistry and molecular biology,* 40 (10), 699-712.

Boone, C.K., Six, D.L., Zheng, Y. and Raffa, K.F., (2008). Parasitoids and dipteran predators exploit volatiles from microbial symbionts to locate bark beetles. *Environmental Entomology,* 37 (1), 150-61.

Boone, C.K., Aukema, B.H., Bohlmann, J., Carroll, A.L. and Raffa, K.F., (2011). Efficacy of tree defense physiology varies with bark beetle population density: a basis for positive feedback in eruptive species. *Canadian Journal of Forest Research,* 41 (6), 1174-88.

Boone, C.K., Keefover-Ring, K., Mapes, A.C., Adams, A.S., Bohlmann, J. and Raffa, K.F., (2013). Bacteria associated with a tree-killing insect reduce concentrations of plant defense compounds. *Journal of chemical ecology,* 39 (7), 1003-06.

Bras, A., Avtzis, D.N., Kenis, M., Li, H., Vétek, G., Bernard, A., Courtin, C., Rousselet, J., Roques, A. and Auger-Rozenberg, M.A., (2019). A complex invasion story underlies the fast spread of the invasive box tree moth (*Cydalima perspectalis*) across Europe. *Journal of Pest Science 92,* pp.1187-1202.

Breer, H., (1997). Molecular mechanisms of pheromone reception in insect antennae. *Insect pheromone research: New directions*, pp.115-130.

Buotte, P.C., Hicke, J.A., Preisler, H.K., Abatzoglou, J.T., Raffa, K.F. and Logan, J.A., (2016). Climate influences on whitebark pine mortality from mountain pine beetle in the Greater Yellowstone Ecosystem. *Ecological Applications,* 26 (8), 2507-24.

Cheney, B.,(2007). Introduction to scanning electron microscopy. Materials Engineering department San Jose State University.

Christiansen, E.and Bakke, A., (1988). The spruce bark beetle of Eurasia. *Dynamics of forest insect populations*, 479-503.

Cocuzza, G.E.M., Di Silvestro, S., Giordano, R. and Rapisarda, C., (2015). Congruence between cytochrome oxidase I (COI) and morphological data in Anuraphis spp.(Hemiptera, Aphididae) with a comparison between the utility of the 5'barcode and 3'COI regions. *ZooKeys,* (529), 123.

De Bruyne, M., Clyne, P.J., and Carlson, J.R., (1999). Odor coding in a model olfactory organ: The *Drosophila* maxillary palp. *Journal of Neuroscience,* 19 (11), 4520-32.

De Bruyne, M., Foster, K., and Carlson, J.R., (2001). Odor coding in the Drosophila antenna. *Neuron,* 30 (2), 537-52.

De Leon, D., (1935). A study of *Medetera aldrichii* Wh.(Diptera—Dolichopodidae), a predator of the mountain pine beetle (Dendroctonus monticolae Hopk., Coleo.—Scolytidae). *Entomol. Am,* 15 (2), 59-91.

Dippel, C., Heidger, C., Nicolai, V. and Simon, M., (1997). The influence of four different predators on bark beetles in European forest ecosystems (Coleoptera: Scolytidae). *Entomologia generalis,* 21 (3), 161-75.

Duan, Q., Bonn, B., and Kreuzwieser, J., (2020). Terpenoids are transported in the xylem sap of Norway spruce. *Plant, Cell & Environment,* 43 (7), 1766-78.

Elmore, T., Ignell, R., Carlson, J.R. and Smith, D.P., (2003). Targeted mutation of a *Drosophila* odor receptor defines receptor requirement in a novel class of sensillum. *Journal of Neuroscience,* 23 (30), 9906-12.

Evans, H.F., and Fielding, N.J., (1994). Integrated management of *Dendroctonus micans* in the UK. *Forest ecology and management,* 65 (1), 17-30.

Faccoli, M., (2002). Winter mortality in sub-corticolous populations of *Ips typographus* (Coleoptera, Scolytidae) and its parasitoids in the south-eastern Alps, Anzeiger für Schädlingskunde. *Journal of pest science,* 75, 62-68.

Farré-Armengol, G., Filella, I., Llusia, J. and Peñuelas, J., (2016) Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions. *Trends in Plant Science* 21, 10: 854-860.

Fettig, Christopher J, et al. (2022). Ecological consequences of mountain pine beetle outbreaks in the Intermountain West. Forest Health Monitoring: national status, trends, and analysis 2021. Gen. Tech. Rep. SRS-266. Asheville, NC: US Department of Agriculture Forest Service, Southern Research Station: *167–175,* 2022, 167-75.

Fitzgerald, T.D., and Nagel, W.P., (1972). Oviposition and larval bark-surface orientation of *Medetera aldrichii* (Diptera: Dolichopodidae): response to a prey-liberated plant terpene. *Annals of the Entomological Society of America,* 65 (2), 328-30.

Goheen, D.J., and Hansen, E.M., (1993). Effects of pathogens and bark beetles on forests.

Goyer, R.A., Lenhard, G.J., and Strom, B.L., (2004). The influence of silhouette color and orientation on arrival and emergence of Ips pine engravers and their predators in loblolly pine. *Forest Ecology and Management,* 191 (1), 147-55.

Grégoire, J.C., Couillien, D., Drumont, A., Meyer, H. and Francke, W., (1992). Semiochemicals and the management of *Rhizophagus grandis* Gyll.(Col., Rhizophagidae) for the biocontrol of *Dendroctonus micans* Kug.(Col., Scolytidae). *Journal of Applied Entomology,* 114 (1-5), 110-12.

Hajek, A.E., and Eilenberg, J., (2018). Natural enemies: an introduction to biological control (Cambridge University Press).

Hammerbacher, A., Schmidt, A., Wadke, N., Wright, L.P., Schneider, B., Bohlmann, J., Brand, W.A., Fenning, T.M., Gershenzon, J. and Paetz, C., (2013). A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce. *Plant physiology,* 162 (3), 1324-36.

Hansson, B.S., and Stensmyr, M.C., (2011). Evolution of insect olfaction. *Neuron,* 72 (5), 698-711.

Hebert, P.D.N., Ratnasingham, S., and De Waard, J.R., (2003a). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related

species. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 270 (suppl_1), S96-S99.

Hebert, P.D., Cywinska, A., Ball, S.L. and DeWaard, J.R., Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 270 (1512), 313-21.

Hedgren, P.O., and Schroeder, L.M., (2004). Reproductive success of the spruce bark beetle *Ips typographus* (L.) and occurrence of associated species: a comparison between standing beetle-killed trees and cut trees. *Forest Ecology and Management,* 203 (1), 241-50.

Hill, S.R., Hansson, B.S., and Ignell, R., (2009). Characterization of antennal trichoid sensilla from female southern house mosquito, *Culex quinquefasciatus* Say. *Chemical Senses,* 34 (3), 231-52.

Hlásny, T., Krokene, P., Liebhold, A., Montagné-Huck, C., Müller, J., Qin, H., Raffa, K., Schelhaas, M., Seidl, R., Svoboda, M. and Viiri, H., 2019. *Living with bark beetles: impacts, outlook and management options* (No. 8). European Forest Institute.

Hlásny, T., König, L., Krokene, P., Lindner, M., Montagné-Huck, C., Müller, J., Qin, H., Raffa, K.F., Schelhaas, M.J., Svoboda, M. and Viiri, H., (2021). Bark beetle outbreaks in Europe: state of knowledge and ways forward for management. *Current Forestry Reports,* 7, 138-65.

Hopping, G.R., (1947). Notes on the seasonal development of *Medetera Aldrichii* Wheeler (Diptera: Dolichopidae) as a predator of the Douglas fir bark-beetle, *Dendroctonus Pseudotsugae* Hopkins (1). *The Canadian Entomologist,* 79 (7-8), 150-53.

Horntvedt, R., Christiansen, E., Solheim, H. and Wang, S., (1983). Artificial inoculation with *Ips typographus*-associated blue stain fungi can kill healthy Norway spruce trees. *Meddelelser fra Skogforsk*.

Hulcr, J., Ubik, K., and Vrkoc, J., (2006). The role of semiochemicals in tritrophic interactions between the spruce bark beetle *Ips typographus*, its predators and infested spruce. *Journal of Applied Entomology,* 130 (5), 275-83.

Hulcr, J., Pollet, M., Ubik, K. and Vrkoc, J., (2005). Exploitation of kairomones and synomones by *Medetera* spp.(Diptera: Dolichopodidae), predators of spruce bark beetles. *European Journal of Entomology,* 102 (4), 655-62.

Kamińska, A., Lisiewicz, M., Kraszewski, B. and Stereńczak, K., (2021). Mass outbreaks and factors related to the spatial dynamics of spruce bark beetle (*Ips typographus*) dieback considering diverse management regimes in the Białowieża forest. *Forest Ecology and Management,* 498, 119530.

Karamanoli, K., Kokalas, V., Koveos, D. S., Junker, R. R., and Farré-Armengol, G. (2020). Bacteria affect plant-mite interactions via altered scent emissions. *Journal of chemical ecology*, *46*, 782-792.

Kandasamy, D., Gershenzon, J., and Hammerbacher, A., (2016). Volatile organic compounds emitted by fungal associates of conifer bark beetles and their potential in bark beetle control. *Journal of chemical ecology,* 42 (9), 952-69.

Kandasamy, D., Zaman, R., Nakamura, Y., Zhao, T., Hartmann, H., Andersson, M.N., Hammerbacher, A. and Gershenzon, J., (2021). Bark beetles locate fungal symbionts by detecting volatile fungal metabolites of host tree resin monoterpenes. *bioRxiv*, 2021.07. 03.450988.

Kandasamy, D., Zaman, R., Nakamura, Y., Zhao, T., Hartmann, H., Andersson, M.N., Hammerbacher, A. and Gershenzon, J., (2023). Conifer-killing bark beetles locate fungal symbionts by detecting volatile fungal metabolites of host tree resin monoterpenes. *PLoS biology,* 21 (2), e3001887.

Kausrud, K., Økland, B., Skarpaas, O., Grégoire, J.C., Erbilgin, N. and Stenseth, N.C., (2012). Population dynamics in changing environments: the case of an eruptive forest pest species. *Biological Reviews,* 87 (1), 34-51.

Keeling, C.I., and Bohlmann, J., (2006). Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytologist,* 170 (4), 657-75.

Kenis, M., Hurley, B.P., Hajek, A.E. and Cock, M.J., (2017). Classical biological control of insect pests of trees: facts and figures. *Biological Invasions,* 19, 3401-17.

Kenis, M., Hurley, B.P., Colombari, F., Lawson, S., Sun, J., Wilcken, C., Weeks, R. and Sathyapala, S., (2019). Guide to the classical biological control of insect pests in planted and natural forests. *FAO Forestry Paper,* (182).

Kirisits, T., (2004). Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. *Bark and wood boring insects in living trees in Europe, a synthesis*, 181-236.

Klapwijk, M.J., Bylund, H., Schroeder, M. and Björkman, C., (2016). Forest management and natural biocontrol of insect pests. *Forestry,* 89 (3), 253-62.

Krieger, J. and Breer, H., (1999). Olfactory reception in invertebrates. *Science*, *286*(5440), pp.720-723.

Krokene, P., (2015). Conifer defense and resistance to bark beetles. *Bark beetles* (Elsevier), 177-207.

Lanne, B.S., Ivarsson, P., Johnsson, P., Bergström, G. and Wassgren, A.B., (1989). Biosynthesis of 2-methyl-3-buten-2-ol, a pheromone component of *Ips typographus* (Coleoptera: Scolytidae). *Insect Biochemistry,* 19 (2), 163-67.

Lawson, S.A., Furuta, K., and Katagiri, K., (1996). The effect of host tree on the natural enemy complex of *Ips typographus* japonicus Niijima (Col., Scolytidae) in Hokkaido, Japan. *Journal of Applied Entomology,* 120 (1-5), 77-86.

Leufvén, A., Bergström, G., and Falsen, E., (1984). Interconversion of verbenols and verbenone by identified yeasts isolated from the spruce bark beetle *Ips typographus*. *Journal of Chemical Ecology,* 10 (9), 1349-61.

Leufvén, A., Bergström, G., and Falsen, E. (1988). Oxygenated monoterpenes produced by yeasts, isolated from *Ips typographus* (Coleoptera: Scolytidae) and grown in phloem medium. *Journal of chemical ecology,* 14 (1), 353-62.

Lindström, M., Norin, T., Birgersson, G. and Schlyter, F., (1989). Variation of enantiomeric composition of α-pinene in norway spruce, *Picea abies*, and its influence on production of verbenol isomers by *Ips typographus* in the field. *Journal of chemical ecology,* 15 (2), 541-48.

Lopez-Vaamonde, C., Kirichenko, N., Cama, A., Doorenweerd, C., Godfray, H.C.J., Guiguet, A., Gomboc, S., Huemer, P., Landry, J.F., Laštůvka, A. and Laštůvka, Z., (2021). Evaluating DNA barcoding for species identification and discovery in European gracillariid moths. *Frontiers in Ecology and Evolution*, *9*, p.626752.

MacQuarrie, C.J., Lyons, D.B., Seehausen, M.L. and Smith, S.M., (2016). A history of biological control in Canadian forests, 1882–2014. *The Canadian Entomologist,* 148 (S1), S239-S69.

Marini, L., Økland, B., Jönsson, A.M., Bentz, B., Carroll, A., Forster, B., Grégoire, J.C., Hurling, R., Nageleisen, L.M., Netherer, S. and Ravn, H.P., (2017). Climate drivers of bark beetle outbreak dynamics in Norway spruce forests. *Ecography,* 40 (12), 1426-35.

Martikainen, P., Siitonen, J., Kaila, L., Punttila, P. and Rauh, J., (1999). Bark beetles (Coleoptera, Scolytidae) and associated beetle species in mature managed and old-growth boreal forests in southern Finland. *Forest Ecology and Management,* 116 (1-3), 233-45.

Martin, D.M., Gershenzon, J., and Bohlmann, J., (2003). Induction of volatile terpene biosynthesis and diurnal emission by methyl jasmonate in foliage of Norway spruce. *Plant physiology,* 132 (3), 1586-99.

Meiklejohn, K.A., Wallman, J.F., and Dowton, M., (2013). DNA barcoding identifies all immature life stages of a forensically important flesh fly (Diptera: Sarcophagidae). *Journal of Forensic Sciences,* 58 (1), 184-87.

Mills, N.J., (1986). A preliminary analysis of the dynamics of within tree populations of *Ips typographus* (L.)(Coleoptera: Scolytidae). *Journal of Applied Entomology,* 102 (1-5), 402-16.

Montagné-Huck, C., and Brunette, M., (2018). Economic analysis of natural forest disturbances: A century of research. *Journal of Forest Economics,* 32, 42-71.

Morris, J.L., Cottrell, S., Fettig, C.J., Hansen, W.D., Sherriff, R.L., Carter, V.A., Clear, J.L., Clement, J., DeRose, R.J., Hicke, J.A. and Higuera, P.E., (2017). Managing bark beetle impacts on ecosystems and society: priority questions to motivate future research. *Journal of Applied Ecology,* 54 (3), 750-60.

Nagel, W.P., and Fitzgerald, T.D., (1975). *Medetera aldrichii* larval feeding behavior and prey consumption [Dipt.: Dolichopodidae]. *Entomophaga,* 20 (1), 121-27.

Negrobov, O.P., and Naglis, S., (2016). Palaearctic species of the genus Medetera (Diptera: Dolichopodidae). *Zoosystematica Rossica,* 25 (2), 333-79.

Negrobov, O.P., and Von Stackelberg, A.A., (1972). 29. Dolichopodidae. In Lindner, E. (ed.): Die Fliegen der Palaearktischen Region, 4(5), Lief. 289: 257-302.

Negrobov, O.P., and Von Stackelberg,, A.A., (1974a). 29. Dolichopodidae. In Lindner, E. (ed.): Die Fliegen der Palaearktischen Region, 4(5), Lief. 302: 303-324.

Negrobov, O.P., and Von Stackelberg, A.A., (1974b). 29. Dolichopodidae. In Lindner, E. (ed.): Die Fliegen der Palaearktischen Region, 4(5), Lief. 303: 325-346.

Negrobov, O.P., (1977). Dolichopodidae. In Lindner, E. (ed.): Die Fliegen der Palaearktischen Region. 4(5), Lief. 316: 347-386.

Netherer, S., Kandasamy, D., Jirosová, A., Kalinová, B., Schebeck, M. and Schlyter, F., (2021). Interactions among Norway spruce, the bark beetle *Ips typographus* and its fungal symbionts in times of drought. *Journal of Pest Science,* 94 (3), 591-614.

Nicolai, V., (1995). The impact of *Medetera dendrobaena* Kowarz (Dipt., Dolichopodidae) on bark beetles. *Journal of Applied Entomology,* 119 (1-5), 161-66.

Nuorteva, M., (1956). Über den Fichtenstamm-Bastkäfer, *Hylurgops palliatus* Gyll., und seine Insektenfeinde (13: Sanoma Oy).

Paine, T.D., Raffa, K.F., and Harrington, T.C., (1997). Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annual review of entomology,* 42 (1), 179-206.

Pérez-Delgado, A.J., Arribas, P., Hernando, C., López, H., Arjona, Y., Suárez-Ramos, D., Emerson, B.C. and Andújar, C., (2022). Hidden island endemic species and their implications for cryptic speciation within soil arthropods. *Journal of Biogeography*, *49*(7), pp.1367-1380.

Pettersson, E.M., Sullivan, B.T., Anderson, P., Berisford, C.W. and Birgersson, G., (2000). Odor perception in the bark beetle parasitoid *Roptrocerus xylophagorum* exposed to host associated volatiles. *Journal of Chemical Ecology,* 26 (11), 2507-25.

Pettersson, E.M., (2001). Volatile attractants for three Pteromalid parasitoids attacking concealed spruce bark beetles. *Chemoecology,* 11 (2), 89-95.

Pettersson, E.M., and Boland, W., (2003). Potential parasitoid attractants, volatile composition throughout a bark beetle attack. *Chemoecology,* 13 (1), 27-37.

Pollet, M., Germann, C., and Bernasconi, M.V., (2011). Phylogenetic analyses using molecular markers reveal ecological lineages in *Medetera* (Diptera: Dolichopodidae). *The Canadian Entomologist,* 143 (6), 662-73.

Pollet, M., Andrade, R., Gonçalves, A., Álvarez Fidalgo, P., Camaño Portela, J.L., Belin, F., Mortelmans, J. and Stark, A., (2022). Discovery of a Lineage of Soil-Dwelling *Medetera* Species with Multi-Coloured Eyes in Southern Europe (Diptera: Dolichopodidae). *Insects,* 13 (11), 1012.

Powell, C., Caleca, V., Sinno, M., van Staden, M., van Noort, S., Rhode, C., Allsopp, E. and van Asch, B., (2019). Barcoding of parasitoid wasps (Braconidae

and Chalcidoidea) associated with wild and cultivated olives in the Western Cape of South Africa. *Genome,* 62 (3), 183-99.

Qiu, Y.T., van Loon, J.J., Takken, W., Meijerink, J., and Smid, H.M., (2006). Olfactory coding in antennal neurons of the malaria mosquito, *Anopheles gambiae*. *Chemical senses,* 31 (9), 845-63.

Raffa, K.F., Gregoire, J.C., and Lindgren, B.S., (2015). Natural history and ecology of bark beetles. *Bark Beetles* (Elsevier), 1-40.

Raffa, K.F., Aukema, B.H., Bentz, B.J., Carroll, A.L., Hicke, J. A., Turner, M.G., and Romme, W.H., (2008). Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. *Bioscience,* 58 (6), 501-17.

Schlyter, F., Birgersson, G., and Leufvén, A., (1989). Inhibition of attraction to aggregation pheromone by verbenone and ipsenol. *Journal of Chemical Ecology,* 15 (8), 2263-77.

Schlyter, F., Birgersson, G., Byers, J. A., Löfqvist, J., and Bergström, G., (1987a). Field response of spruce bark beetle, *Ips typographus,* to aggregation pheromone candidates. *Journal of chemical ecology,* 13 (4), 701-16.

Schlyter, F., Löfqvist, J. & Byers J.A., (1987b). Behavioural sequence in the attraction of the bark beetle *Ips typographus* to pheromone sources, Physiological Entomology, 12: 185-196.

Schneider, D., (1964). Insect antennae. *Annual review of entomology,* 9 (1), 103-22.

Seidl, R., and Rammer, W., (2017). Climate change amplifies the interactions between wind and bark beetle disturbances in forest landscapes. *Landscape Ecology,* 32, 1485-98.

Seidl, R., Rammer, W., Jäger, D., and Lexer, M. J., (2008). Impact of bark beetle (Ips typographus L.) disturbance on timber production and carbon sequestration in different management strategies under climate change. *Forest Ecology and Management,* 256 (3), 209-20.

Seidl, R., Schelhaas, M. J., Rammer, W., and Verkerk, P.J., (2014). Increasing forest disturbances in Europe and their impact on carbon storage. *Nature climate change,* 4 (9), 806-10.

Sousa, M., Ignell, R., Pollet, M., Green, K.K., Becher, P.G., and Birgersson, G., (2023a). Antennal and maxillary palp morphology, and sensillar equipment, of the spruce bark beetle predators, *Medetera signaticornis* and *Medetera infumata* (Diptera: Dolichopodidae). *Arthropod Structure & Development,* 72, 101229.

Sousa, M., Birgersson, G., Karlsson Green, K., Pollet, M., and Becher, P.G., (2023b) Odors Attracting the Long-Legged Predator *Medetera* signaticornis Loew to *Ips typographus* L. Infested Norway Spruce Trees. *Journal of Chemical Ecology*, 1-14.

Stenberg, J.A., Sundh, I., Becher, P.G., Björkman, C., Dubey, M., Egan, P.A., (2021). When is it biological control? A framework of definitions, mechanisms, and classifications. *Journal of Pest Science,* 94 (3), 665-76.

Stephen, F.M., and Dahlsten, D.L., (1976). The arrival sequence of the arthropod complex following attack by *Dendroctonus brevicomis* (Coleoptera: Scolytidae) in ponderosa pine. *The Canadian Entomologist,* 108 (3), 283-304.

Ståhls, G., Vujic, A., Pérez-Bañon, C., Radenkovic, S., Rojo, S., & Petanidou, T., (2009). COI barcodes for identification of *Merodon* hoverflies (Diptera, Syrphidae) of Lesvos Island, Greece. *Molecular ecology resources,* 9 (6), 1431-38.

Thom, D. and Seidl, R., (2016). Natural disturbance impacts on ecosystem services and biodiversity in temperate and boreal forests. *Biological Reviews,* 91 (3), 760-81.

Tømmerås, B.Å., (1985). Specialization of the olfactory receptor cells in the bark beetle *Ips typographu*s and its predator *Thanasimus formicarius* to bark beetle pheromones and host tree volatiles. *Journal of Comparative Physiology A,* 157 (3), 335-41.

Ulrich, H., (2004). Predation by adult Dolichopodidae (Diptera): a review of literature with an annotated prey-predator list. *Studia dipterologica,* 11 (2), 369-403.

Vité, J.P., and Pitman, G.B., (1969). Aggregation behaviour of *Dendroctonus brevicomis* in response to synthetic pheromones. *Journal of insect physiology,* 15 (9), 1617-22.

Wallin, K.F., and Raffa, K.F., (2004). Feedback between individual host selection behavior and population dynamics in an eruptive herbivore. *Ecological monographs,* 74 (1), 101-16.

Waterhouse DF & Sands DPA. 2001. Classical biological control ofarthropods in Australia. ACIAR Monograph No. 77, Canberra, Australia.

Wegensteiner, R., Wermelinger, B., and Herrmann, M., (2015). Natural enemies of bark beetles: predators, parasitoids, pathogens, and nematodes, *Bark Beetles* (Elsevier), 247-304.

Wermelinger, B., (2002), Development and distribution of predators and parasitoids during two consecutive years of an *Ips typographus* (Col., Scolytidae) infestation, *Journal of Applied Entomology,* 126 (10), 521-27.

Wermelinger, B., Epper, C., Kenis, M., Ghosh, S., & Holdenrieder, O., (2012). Emergence patterns of univoltine and bivoltine *Ips typographus* (L.) populations and associated natural enemies. *Journal of applied entomology,* 136 (3), 212-24.

Wermelinger, B., (2004). Ecology and management of the spruce bark beetle *Ips typographus*—a review of recent research, *Forest ecology and management,* 202 (1), 67-82.

Wermelinger, B., and Jakoby, O., (2022). Bark Beetles, in Thomas Wohlgemuth, Anke Jentsch, and Rupert Seidl (eds.). *Disturbance Ecology*. Cham: Springer International Publishing, 271-93.

Weslien, J., and Regnander, J., (1992). The influence of natural enemies on brood production in *Ips typographus* (Col. Scolytidae) with special reference to

egg-laying and predation by *Thanasimus formicarius* (Col.: Cleridae). *Entomophaga,* 37 (2), 333-42.

Weslien, J., (1992). The arthropod complex associated with *Ips typographus* (L.) (Coleoptera, Scolytidae): species composition, phenology, and impact on bark beetle productivity. *Entomologica Fennica,* 3 (4), 205-13.

Wichmann, L., and Ravn, H.P., (2001). The spread of *Ips typographus* (L.)(Coleoptera, Scolytidae) attacks following heavy windthrow in Denmark, analysed using GIS. *Forest Ecology and Management,* 148 (1-3), 31-39.

Williamson, D.L., (1971). Olfactory Discernment of Prey by *Medetera bistriata* (Diptera: Dolichopodidae). *Annals of the Entomological Society of America,* 64 (3), 586-89.

Yang, D., Zhu, Y., Wang, M., Zhang, L., (2006). World Catalogue of Dolichopodidae (Insecta: Diptera). China Agric. Univ. Beijing, pp. 1e704.

Zhao, T., Kandasamy, D., Krokene, P., Chen, J., Gershenzon, J., & Hammerbacher, A., (2019). Fungal associates of the tree-killing bark beetle, *Ips tyographus*, vary in virulence, ability to degrade conifer phenolics and influence bark beetle tunneling behavior. *Fungal ecology,* 38, 71-79.

Öhrn, P., Berlin, M., Elfstrand, M., Krokene, P., & Jönsson, A.M., (2021). Seasonal variation in Norway spruce response to inoculation with bark beetle-associated bluestain fungi one year after a severe drought. *Forest Ecology and Management,* 496, 119443.

Popular science summary

In forests, bark beetles are naturally occurring organisms that mostly mate and reproduce on dead or dying trees, where they play an important role in the decomposition and nutrient cycling of wood. However, under warm, dry conditions, some bark beetle species can develop high population densities and are able to attack and kill healthy trees. Such outbreaks cause great economic and ecological damage. Economically, bark beetles can damage large quantities of timber, significantly affecting the forestry industry. Ecologically, intensive outbreaks can affect forest ecosystems and the services they provide, and change entire landscapes. In many managed forests around Europe, several management practices aim at preventing bark beetle outbreaks. These include felling and removal of bark beetle-infested standing trees and removal of windthrown timber that can be used by bark beetles as a breeding site. In combination with these practices, natural enemies can also be used in biological control programmes to regulate the population densities of bark beetles safely and sustainably.

Several species of long-legged flies within the genus *Medetera* are important bark beetle predators. Females of these fly species are attracted to infested trees, where they lay their eggs on the bark surface. Few days later the hatched larvae migrate into the beetle galleries, where they prey on the developmental stages of bark beetles, and can contribute significantly to a high bark beetle mortality. However, very little is known about their species diversity, ecology, and how they locate infested trees. In published literature, I found around thirty *Medetera* species described being associated with tree-killing bark beetles. Some of these species were found on Norway spruce trees in Southern Sweden that were infested with the Eurasian bark beetle *Ips typographus*. There, *M. signaticornis* was the most abundant species. Further analysis revealed that both male and female *M. signaticornis* use

olfaction to find infested trees, and that their antennal “nose” can detect compounds directly produced by bark beetles, bark beetle-associated microorganisms and spruce trees. Trap tests using synthetic blends composed of several of these odour compounds as bait showed that significant numbers of *M. signaticornis* and other *Medetera* species were efficiently trapped. However, these blends still need to be optimised for practical application in forestry.

Populärvetenskaplig sammanfattning

I våra svenska skogar är barkborrar naturligt förekommande insekter, som mestadels förökar sig på redan döda eller döende träd, där de spelar en viktig roll i trädets nedbrytning och näringskretslopp. Under varma och torra somrar kan ett fåtal arter dock nå höga populationstätheter, och kan då komma att attackera och döda även levande träd. Sådana massangrepp kan innebära stora ekonomiska och ekologiska skador. Ekonomiskt sett kan barkborrar skada en stor mängd timmer, och därmed avsevärt påverka skogsindustrin. Ekologiskt sett kan intensiva utbrott påverka skogens ekosystem och de ekosystemtjänster den utför, samt förändra hela landskap. I många produktionsskogar runt om i Europa används skötselmetoder med syfte att förhindra utbrott av barkborrar. Dessa inkluderar fällning och borttagning av barkborreangripna stående träd, samt borttagning av vindfällda träd, vilka kan användas av barkborrarna som yngelmaterial. I kombination med dessa metoder kan även barkborrarnas naturliga fiender användas i biologiska kontrollprogram för att på ett säkert och hållbart sätt reglera mängden barkborrar i våra skogar.

Flera arter av styltflugor av släktet *Medetera* är viktiga rovdjur på barkborrarna. Honor av dessa flugarter attraheras av angripna träd, där de lägger sina ägg på barken. Några dagar senare vandrar de kläckta larverna in i skalbaggarnas gångar där de jagar barkborrar av alla utvecklingsstadier och väsentligen kan bidra till en hög dödlighet för barkborrarna. Mycket lite är dock känt om deras mångfald och ekologi, och om hur de lokaliserar barkborreangripna träd. I den vetenskapliga litteraturen hittade jag trettio beskrivna arter av *Medetera* som är förknippade med träddödande barkbaggar. En del av dessa arter hittades på granar angripna av granbarkborren, *Ips typographus*, i södra Sverige, varav *M. signaticornis* var den vanligast förekommande arten. Vidare studier visade också att både

hanar och honor av *M. signaticornis* använder dofter för att hitta de angripna träden och att deras antenner (”näsan”) kan upptäcka kemiska substanser producerade av barkborrarna själva, av mikroorganismer som är associerade med barkborrarna och av granar. Fältförsök visade att ett betydande antal *M. signaticornis*, och andra *Medetera*-arter, effektivt kunde fångas med fällor som betats med syntetiska blandningar bestående av ett flertal av dessa doftämnen. Dessa blandningar måste dock fortfarande optimeras ytterligare innan de kan komma till praktisk användning i skogsindustrin.

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II

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Antennal and maxillary palp morphology, and sensillar equipment, of the spruce bark beetle predators, *Medetera signaticornis* and *Medetera infumata* (Diptera: Dolichopodidae)

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ABSTRACT

Many long-legged *Medetera* flies are natural enemies of bark beetle pests, which they detect using olfactory cues, likely through olfactory sensilla on the antennae and maxillary palps. Morphological characterisation of olfactory sensilla among insects can provide a basis for future taxonomic, phylogenetic or electrophysiological studies. Scanning electron microscopy was used to describe the morphology of olfactory organs and sensillar equipment of *Medetera signaticornis* and *M. infumata*. Three different olfactory sensillum types were found in both fly species, sensilla trichodea, s. basiconica and grooved pegs. Based on size and wall structure, s. trichodea and s. basiconica were categorised into different subtypes. Sharp-tipped curved s. trichodea, and small, large and thin s. basiconica were found on the antennal postpedicel of *M. signaticornis* adults, while grooved s. basiconica were found in *M. infumata*. The density of sharp-tipped long s. trichodea was significantly higher in males compared to females, and in *M. signaticornis* compared to *M. infumata*. Long-grooved s. basiconica were found grouped in a small pit on the maxillary palps of both species. Comparison of our results with the limited available ecological data suggests that differences in numbers of specific sensillum types may reflect adaptations related to olfactory-driven behaviours such as host seeking.

1. Introduction

Medetera (Fischer von Waldheim, 1819) is by far the genus most rich in species within the subfamily Medeterinae (Diptera: Dolichopodidae). The genus includes over 300 species worldwide (Yang et al., 2006), with approximately 190 species reported from the Palaearctic realm, where it also reaches its highest species richness (Bickel, 1985; Pollet, 2011). Most flies of this genus are small (1.5–4.5 mm), and feature a metallic greenish to bluish body colour covered with small hair-like structures. Adults are commonly found on vertical substrates such as tree trunks, rocks or walls, while larvae are mainly confined to subcortical galleries of dead or weakened living trees (De Leon, 1935; Nuorteva, 1956; Pollet et al., 2011). Both adults and larvae are predators of a wide variety of small soft-bodied arthropods (Bickel, 1985). Several *Medetera* species are known to prey on early developmental stages of tree-killing bark beetles (Coleoptera: Curculionidae, Scolytinae) that are significant pests of coniferous forests (Beaver, 1966). As such, these species are considered as one of the most important natural enemies of bark beetle populations, and have the potential to be used for biological pest management (Dippel et al., 1997; Wermelinger, 2002; Hedgren and Schroeder, 2004). However, little is known about their life history, ecology and how they locate their prey. Previous studies have suggested that *Medetera* species use odorant chemical signals to locate spruce trees attacked by *Ips typographus* Linnaeus, 1758 (Hulcr et al., 2005, 2006), but the morphology of their peripheral olfactory organs and sensillar equipment is yet to be described.

The main olfactory organs in adult insects, i.e., the antennae, maxillary and labial palps, house various types of hair-like structures called sensilla, which are involved in the detection of chemical, mechanical and thermo-hygro stimuli in the environment (Schneider, 1964; Hansson and Stensmyr, 2011). These sensilla can be

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classified into different types based on their external wall-structure, pore density, shape, size and number of enclosed sensory neurons (Hallberg and Hansson, 1999; Lin and Potter, 2015). The types and abundance of these sensilla vary across insect species. Such variation in the morphology of the olfactory organs often reflects adaptations, both between species and sexes, to locate for example prey, habitat or mates (Hansson and Stensmyr, 2011). The arrangement of the olfactory organs and the diversity within the sensillar equipment can thus be used for taxonomic, phylogenetic or evolutionary ecological studies (Zhang et al., 2016). In addition, structural characterisation of the olfactory organs can provide the basis for future functional classification of olfactory sensory neurons, present within various sensillum types (De Bruyne et al., 1999, 2001).

In this study, we used scanning electron microscopy (SEM) in order to compare and describe the antennal morphology and sensillar equipment of two bark beetle predators, *Medetera signaticornis* Loew, 1857 and *M. infumata* Loew, 1857. We focused on these two species because they were the two most common species that were collected from bark beetle-infested trees during our field work. We hypothesized that flies of both species may display a similar morphology of their olfactory organs and sensillar equipment. The motivation for such hypothesis is because both species co-exist on coniferous trees, are known to oviposit on bark beetle-infested Norway spruce trees, and appear to be attracted by the spruce bark beetle pheromones (Hulcr et al., 2005; Wegensteiner et al., 2015).

2. Material and methods

2.1. Field collections of adult flies

The study material, males and females of *M. signaticornis* and *M. infumata*, was collected at two locations in Asa, Sweden (57.150°N, 14.765°E, and 57.127°N, 14.780°E, respectively) between May–August during 2018 and 2019. Adult flies were collected with a mouth aspirator from bark beetle-infested Norway spruce trees (*Picea abies*), and kept in individual vials with a humidified filter paper to prevent fly tissue dehydration, until further analysis.

2.2. Scanning electron microscopy

To prepare the antennae and the maxillary palps for SEM, the heads of the flies were detached from the bodies. Subsequently, the heads were immersed overnight in a fixative solution (2% paraformaldehyde, Tab Laboratory equipment, Aldermaston, UK) and 2.5% glutaraldehyde (Agar Scientific, Essex, UK), and washed in 0.1 M sodium cacodylate buffer (Agar Scientific, Essex, UK), with pH adjusted to 7.3. After fixation, each fly head was dehydrated in a graded series of ethanol (75%, 96% and 100% twice, for 15 min), followed by critical-point drying (030 Bal-Tec Inc, EM-Technology and Application, Büntle, Lichtenstein), using liquid carbon dioxide (CO_2). To obtain a full three-dimensional view of the antennae and the maxillary palps, heads were carefully affixed in frontal view on aluminium stubs with double-sided adhesive tape. Then, to increase conductivity, the dried heads were sputter-coated with gold ions (Sputter coater 108 auto, Cressington Scientific Instruments, Watford, UK) at 20 mA for 65 s. Imaging was performed with a high-resolution scanning electron microscope (SU3500, Hitachi high-tech, Tokyo, Japan) at 5 kV, at a working distance of 5–15 mm.

2.3. The antennal and maxillary palp morphology and sensillar equipment

For morphological measurements and sensillar counts in each species, six individuals from each sex were used. However, only the heads of the specimens that allowed a good lateral view of the inner part of the antennal postpedicel (i.e., the section directed towards the medial body plane) and the outer part of the maxillary palps were included in the analysis, i.e., *M. signaticornis:* females (n = 6) and males (n = 5); *M. infumata:* females (n = 5) and males (n = 4). For each individual, the length of the arista-like stylus, and the length and widths of the postpedicel and palp were recorded. Then, the lateral view of the inner part of the postpedicel and outer part of the palp was magnified, and the different morphological sensillum types were identified and counted. Furthermore, a subset of all sensillum types was selected for measurements of their length and basal width. All comparisons were done with similar magnifications.

To compare the sensillum density between adults of *M. signaticornis,* which are larger in body size compared to *M. infumata*, the surface area of the postpedicel and palps was estimated, similar to Pitts and Zwiebel (2006). For each specimen, the basic formula of an ellipse (r1*r2 *π) was used, in order to estimate the surface area of the inner part of the postpedicel and outer part of maxillary palps. The largest and the smallest diameters of the postpedicel or maxillary palps, respectively, were measured and half their lengths defined as r1 and r2. Then, the sensillum density on the postpedicel and maxillary palps was calculated by dividing the total number of sensilla by the surface area, in which the particular type of sensillum was found. Two-way analysis of variance (ANOVA) was used to compare the relative measurements of the *(i)* length of the arista-like stylus, (*ii*) the surface area of the postpedicel and palp; and *(iii)* the densities of the different sensillum types. Pairwise multiple-comparison post-hoc tests were carried out for the multiple comparisons between sexes, species and statistical interaction between these two variables (sex*species). Statistical analyses were performed using *aov ()* and *Emmeans ()* functions from R studio (version 3.6.1; RStudio-Team, 2018).

3. Results

The criteria for identification and classification of the antennal morphology and sensilla types in this study followed those of Shanbhag et al. (1999), Hallberg and Hansson (1999) and Keil (1999).

3.1. General morphology of the olfactory organs

3.1.1. Antennae

The antennae of adult *M. signaticornis* and *M. infumata* species are located frontally between the large compound eyes (Fig. 1A and B). Each antenna consists of three distinct segments: the scape, the pedicel and the postpedicel with an arista-like stylus (Fig. 1A and B). The postpedicel is oval in shape, and the surface area is significantly larger ($F = 56.5$; $df = 1, 16$; $P < 0.001$) in *M. signaticornis* compared to *M. infumata* (Fig. 1C and D). However, no significant differences in size are observed between sexes ($F = 1.6$; $df = 1, 16$; $P > 0.05$), and neither do the variables of sex and species statistically interact ($F = 1.4$; $df = 1, 16$; $P > 0.05$; Table 1). The terminal arista-like stylus is rigid and long (400 μm–480 μm), and it is located at the end (top) of the postpedicel (Fig. 2A–C). No significant differences are observed in the relative length of the terminal arista-like stylus between sexes ($F = 0.4$; $df = 1, 17$; $P > 0.05$) or species ($F = 1.0$; $df = 1, 17$; $P > 0.05$), nor do the variables of sex and species influence each other by statistical interaction ($F = 0.4$; $df = 1, 17$; $P > 0.05$). This indicates that the lengths of the arista-like stylus are similar between individuals of the different sexes and species.

In both species, a large number of non-innervated microtrichia (Shanbhag et al., 1999) are found on the scape, pedicel, postpedicel

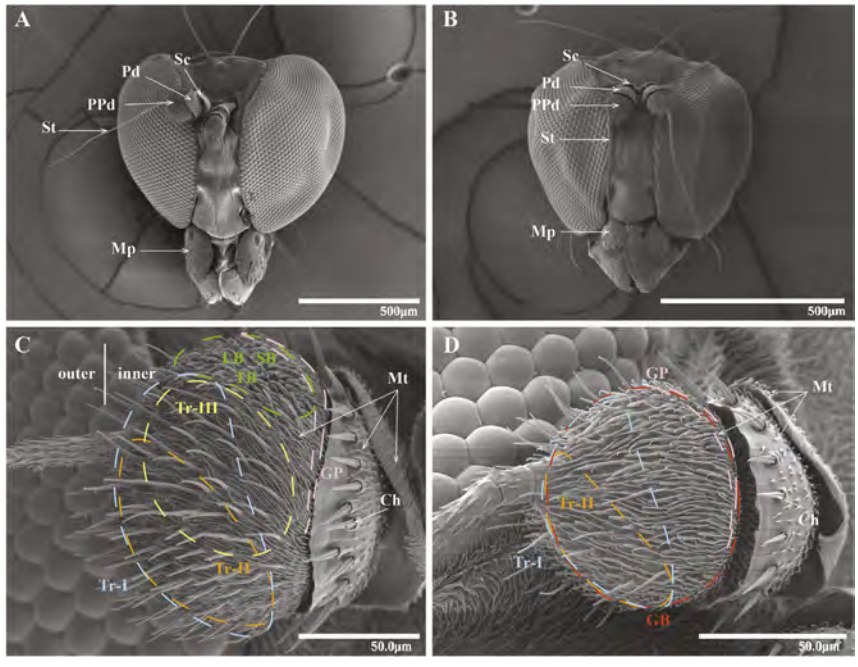

Fig. 1. Morphology of the antenna, maxillary palps and the sensillar arrangement in *Medetera signaticornis* and *Medetera infumata*. **A.** Frontal view of the heads of female *M. signaticornis* and **B.** *M. infumata*, respectively. **C.** Lateral view of the inner part of the postpedicel of female *M. signaticornis* and **D.** *M. infumata*, respectively. Abbreviations: Sc, scape; Pd, pedicel; PPd, postpedicel; St, arista-like stylus; Mp, maxillary palps; LB, large s. basiconica; SB, small s. basiconica; TB, thin s. basiconica and GB, grooved s. basiconica; Tr-I, long s. trichodea; Tr-II, short s. trichodea and Tr-III, curved s. trichodea; GP, grooved pegs; Ch, s. chaetica and Mt, microtrichia. Colored dashed lines indicate the areas where the specific types and subtypes of olfactory sensilla are found.

Table 1
Lengths (µm) of the arista-like stylus and surface areas (µm^2) of the inner part of the postpedicel and the outer part of the maxillary palps are shown as means ± standard errors. Data were analysed for significant differences dependent on sex or species, as well as for statistical interaction between these two variables. Different small letters (a,b,c) following the measures of length and surface area indicate significant differences between sexes or species according to post-hoc tests following two-way ANOVA. F-values provide a measure for variation between samples, df abbreviates the degrees of freedom and $P < 0.05$ provides a measure for significant difference between means.

	M. signaticornis		*M. infumata*		Variables		
	Females	Males	Females	Males	Sex	Species	Sex*Species
Length of the arista-like stylus	472.7 ± 28.4	438.3 ± 11.8	472.0 ± 13.7	465.0 ± 12.9	F = 1.0; df = 1, 17; P > 0.05	F = 0.4; df = 1, 17; P > 0.05	F = 0.4; df = 1, 17; P > 0.05
Surface area of the postpedicel	10218.1 ± 932.6**a**	8642.8 ± 457.5**a**	4372.4 ± 12.9**b**	4403.3 ± 92.0**b**	F = 1.6; df = 1, 16; P > 0.05	F = 56.5; df = 1, 16; P < 0.001	F = 1.4; df = 1, 16; P > 0.05
Surface area of the maxillary palps	18761.5 ± 2160.2**a**	14789.4 ± 1373.3 **ab**	8154.2 ± 412.2**bc**	6329.1 ± 142.4**c**	F = 3.4; df = 1, 14; P > 0.05	F = 33.9; df = 1, 14; P < 0.001	F = 0.4; df = 1, 14; P > 0.05

and arista-like stylus (Fig. 1C and D; Fig. 2A–F). Besides microtrichia no other structures are found on the scape (Fig. 2D). The pedicel is the only antennal segment that houses mechanosensory bristles known as s. chaetica (Ch) (Fig. 1C and D; Fig. 2 D, G, H). While, the postpedicel features three different putative olfactory sensilla types: s. trichodea, s. basiconica and grooved pegs (GP) (Fig. 1C and D).

3.2. Maxillary palps

The maxillary palps in adults of *M. signaticornis* and *M. infumata* are attached to the side of the basodorsal part of the proboscides (Fig. 1A and B), with both species displaying a similar maxillary palp morphology, in regards to the shape and location of the different sensillum types (Fig. 3A and B). The palp surface area of *M. signaticornis* is significantly larger ($F = 33.9$; $df = 1, 14$; $P < 0.001$) compared to *M. infumata*, and although females have a slightly larger palp area compared to males, the difference is not significant ($F = 3.4$; $df = 1, 14$; $P > 0.05$), and no statistical interaction between sex and species is found, i.e., the larger surface in *M. signaticornis* is independent from any variation between sexes ($F = 0.4$; $df = 1, 14$; $P > 0.05$) (Table 1). Each palp consists of a single oval segment, with two different subtypes of Ch (as described below) distributed from the centre to the distal end (Fig. 3A–D). Few microtrichia are distributed along the proximate rim of the palp and over the edges (Fig. 3A, B, E) and a group of long grooved s. basiconica (MpGB) are found in a pit (Fig. 3A, B, F).

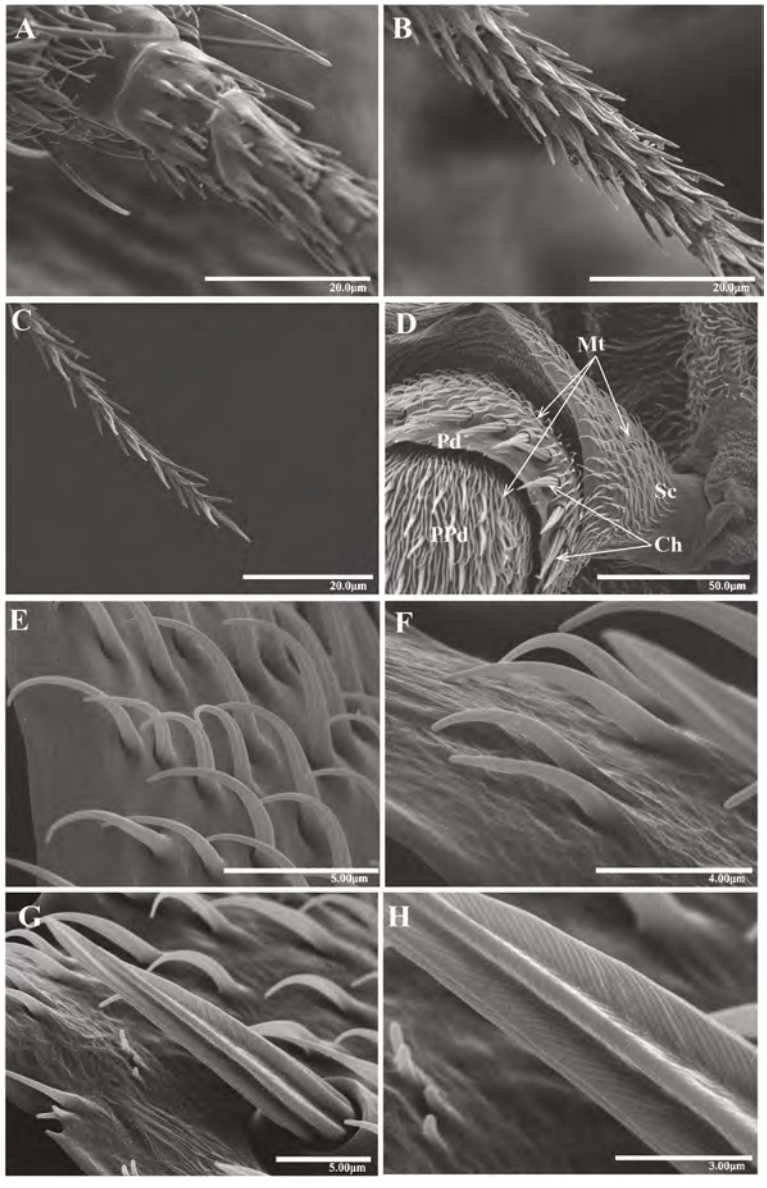

Fig. 2. Arista-like stylus, scape and pedicel of *Medetera signaticornis* and *Medetera infumata*. **A-C**. Different parts (base, middle and tip, respectively) of the arista-like stylus of male *M. infumata*. **D-H.** Pictures from female *M. signaticornis*. **D.** Lateral view of the scape and pedicel. **E-F.** High-resolution of microtrichia from the scape and pedicel, respectively. **G-H.** High-resolution images of s. chaetica from the circumference of the pedicel. Abbreviations: Sc, scape; Pd, pedicel; PPd, postpedicel; Ch, s. chaetica; Mt, microtrichia.

3.3. Sensillum types

3.3.1. Sensilla chaetica (CH)

The Ch arise from a flexible socket, and are cylindrical at the base, and then gradually taper into a long straight hair with a sharp tip. The surface has prominent longitudinal grooves with no discernible wall pores (Fig. 2G and H; Fig. 3C and D). This type of sensillum is present in both species, and is distributed evenly around the circumference of the pedicel (Fig. 1C and D) and from the centre to the distal end of the outer part of the maxillary palp (Fig. 3A and B). According to their length and position, the Ch sensilla on the maxillary palps are classified as Ch-I and Ch-II (Table 2). The density of Ch on the pedicel and Ch-I on the maxillary palps found in *M. infumata* is significantly higher than in

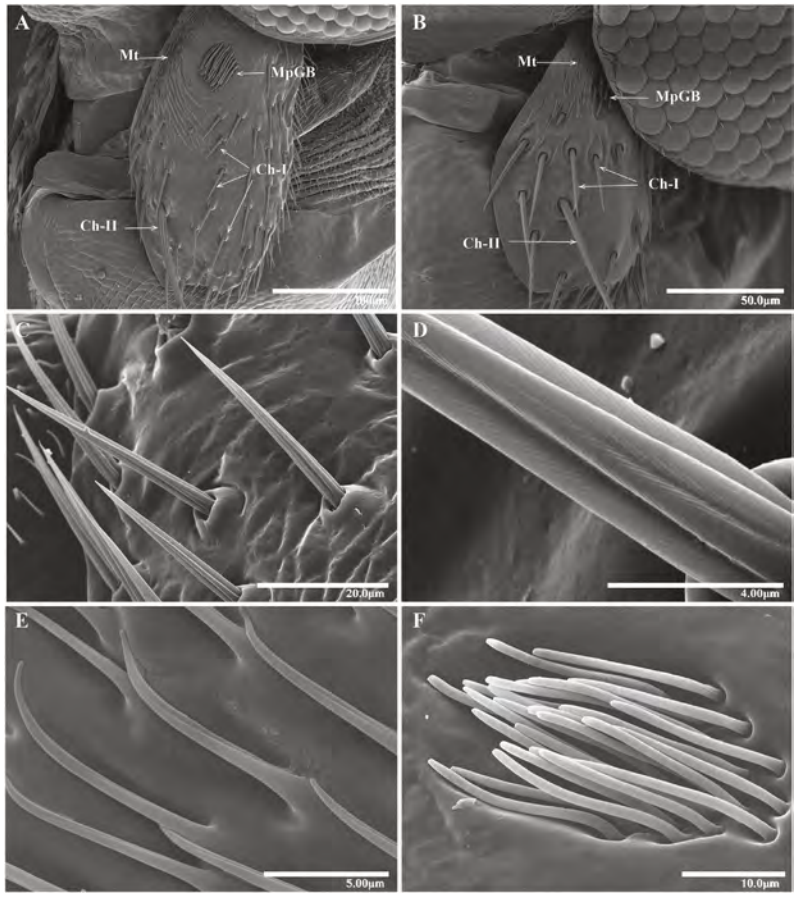

Fig. 3. Morphology of the maxillary palps of *Medetera signaticornis* and *Medetera infumata*. **A-B**. Lateral view of the outer part of maxillary palp of female *M. signaticornis* and *M. infumata*, respectively. **C**–**F.** Pictures from the outer part of the maxillary palp of female *M. signaticornis*. **C-D.** High-resolution of the palpal sensilla chaetica. **E.** High-resolution of microtrichia from the proximate rim of the palp. **F.** Pit with the long grooved s. basionica. Abbreviations: Mt, microtrichia; MpGB, long grooved s. basiconica; Ch-I, s. chaetica subtype I; Ch-II, s. chaetica subtype II.

M. signaticornis (Ch: $F = 71.4$; $df = 1, 13$; $P < 0.001$, Ch-I: $F = 24.7$; $df = 1, 11$; $P < 0.001$), however, no significant differences are observed between sexes (Ch: $F = 0.39$; $df = 1, 13$; $P > 0.05$, Ch-I: $F = 0.9$; $df = 1, 11$; $P > 0.05$) and no statistical interaction is found between the variables of species and sex (Ch: $F = 1.19$; $df = 1, 13$; $P > 0.05$, Ch-I: $F = 4.5$; $df = 1, 11$; $P > 0.05$) (Table 3). Only one long Ch-II sensillum is found on each palp in both species (Fig. 3A and B).

3.4. Sensilla trichodea

Sensilla trichodea are the most abundant sensillum type found on the postpedicel of *M. signaticornis*, and the second most common type found on the postpedicel of *M. infumata* (Fig. 1C and D; Table 2). According to differences in morphology and length, the sharp-tipped s. trichodea may be sub-divided into long (Tr-I), short (Tr-II) and curved s. trichodea (Tr-III) (Fig. 4A–F; Table 2). The Tr-I and Tr-II sensilla are found closely packed along the lateral margin of the postpedicel in both species (Fig. 1 C, D). The Tr-III subtype is only found on the postpedicel of *M. signaticornis*, and is distributed diagonally over the disto-lateral part of both the inner and outer side of the postpedicel. The size of Tr-III is intermediate when compared to Tr-I and Tr-II (Table 2). The cuticle of the Tr-I and Tr-II sensilla is relatively smooth compared to Tr-III, which is characterised by elevated ridges (Fig. 4A–F). All three subtypes have evenly distributed pores and a non-accentuated basal drum. The density of Tr-I found on the postpedicel of *M. signaticornis* is higher compared to *M. infumata* ($F = 21.8$; $df = 1, 16$; $P < 0.001$), and in both cases, males have more Tr-I compared to the females ($F = 13.2$; $df = 1, 16$; $P < 0.01$) (Table 3).

3.5. Sensilla basiconica

The sensilla basiconica are the most common sensillum type found on the postpedicel of *M. infumata* and second most common found on the postpedicel of *M. signaticornis*. According to the morphology and size, these sensilla can be divided into four subtypes; large (LB), small (SB), thin (TB) and grooved (GB) (Fig. 4-G-J)

Table 2
Types, location, numbers and dimensions of sensilla found on the antenna and maxillary palps. Lengths (L) and basal widths (BW) expressed in μm were measured from samples containing males and females and are shown as means ± standard errors. N° represents the numbers of sensilla found on the inner part of the antenna or on the outer part of the maxillary palps. As some types of sensilla are more common than others, different numbers (n) of sensilla were used for the evaluation of L and BW.

Sensilla	Location of the sensilla		*M. signaticornis*					*M. infumata*				
	Antennae	Maxillary palps	N° of sensilla females	N° of sensilla males	L	BW	n	N° of sensilla females	N° of sensilla males	L	BW	n
S. chaetica (Ch)	x		16.5 ± 0.9	15.0 ± 0.6	22.4 ± 1.1	3.3 ± 0.1	15	12.0 ± 0.8	11.5 ± 0.4	22.1 ± 1.5	3.1 ± 0.1	15
S. chaetica subtype I (Ch-I)		x	31.5 ± 1.9	27.5 ± 2.8	31.2 ± 1.6	3.1 ± 0.1	15	17.2 ± 0.4	17.4 ± 1.2	34.0 ± 1.1	2.7 ± 0.1	15
S. chaetica subtype II (Ch-II)		x	1.0 ± 0.0	1.0 ± 0.0	85.0 ± 3.1	7.5 ± 0.4	5	1.0 ± 0.0	1.0 ± 0.0	77.5 ± 2.6	5.0 ± 0.2	5
Long s. trichodea (Tr-I)	x		69.2 ± 3.2	76.6 ± 3.7	23.3 ± 0.4	1.9 ± 0.0	25	24.4 ± 1.8	28.3 ± 0.9	21.2 ± 0.4	1.9 ± 0.0	10
Short s. trichodea (Tr-II)	x		20.2 ± 2.5	14.2 ± 2.0	9.9 ± 0.5	1.9 ± 0.0	12	5.8 ± 1.0	7.5 ± 1.1	10.0 ± 0.3	2.0 ± 0.0	5
Curved s. trichodea (Tr-III)	x		24.5 ± 2.8	28.0 ± 1.2	14.3 ± 0.6	1.8 ± 0.0	12					
Small s. basiconica (SB)	x		27.2 ± 1.6	21.0 ± 3.6	6.7 ± 0.2	1.8 ± 0.0	20					
Large s. basiconica (LB)	x		38.8 ± 3.0	34.0 ± 3.9	4.5 ± 0.2	2.0 ± 0.1	20					
Thin s. basiconica (TB)	x		8.7 ± 0.8	9.2 ± 0.6	8.2 ± 0.3	1.7 ± 0.2	10					
Grooved s. basiconica (GB)	x							47.6 ± 0.6	41.5 ± 2.5	7.6 ± 0.2	1.6 ± 0.0	20
Long grooved s. basiconica (MpGB)		x	31.2 ± 2.2	25.5 ± 1.5	18.6 ± 0.3	1.4 ± 0.1	30	19.5 ± 1.1	12.0 ± 0.5	12.5 ± 0.2	1.9 ± 0.1	30
Grooved pegs (GP)	x		8.5 ± 0.5	8.4 ± 1.0	3.6 ± 0.2	1.6 ± 0.1	10	5.0 ± 0.9	2.3 ± 0.7	3.5 ± 0.3	2.0 ± 0.1	5

Table 3
Sensilla densities (number of sensilla per μm^2 of surface area) found on the inner part of the postpedicel and outer part of the maxillary palps. Data were analysed for significant differences dependent on sex or species, as well as for statistical interaction between these two variables. Different small letters (a,b,c) indicate significant differences between sexes or species according to post-hoc tests following two-way ANOVA. F-values provide a measure for variation between samples, df abbreviates the degrees of freedom and $P < 0.05$ provides a measure for significant difference between means.

Sensilla	*M. signaticornis*		*M. infumata*		Variables		
	Females	Males	Females	Males	Sex	Species	Sex*Species
S. chaetica (Ch)	$1.5 \times 10^{-3} \pm 7.7 \times 10^{-5}$**b**	$1.7 \times 10^{-3} \pm 1.8 \times 10^{-4}$**b**	$2.8 \times 10^{-3} \pm 2.0 \times 10^{-4}$**a**	$2.6 \times 10^{-3} \pm 5.8 \times 10^{-5}$**a**	F = 0.39; df = 1, 13; P > 0.05	F = 71.4; df = 1, 13; P < 0.001	F = 1.19; df = 1, 13; P > 0.05
S. chaetica subtype I (Ch-I)	$1.7 \times 10^{-3} \pm 9.9 \times 10$-^{5}**b**	$1.6 \times 10^{-3} \pm 1.8 \times 10^{-4}$**b**	$2.1 \times 10^{-3} \pm 7.5 \times 10^{-5}$**a**	$2.5 \times 10^{-3} \pm 1.1 \times 10^{-4}$**ab**	F = 0.9; df = 1, 11; P > 0.05	F = 24.7; df = 1, 11; P < 0.001	F = 4.5; df = 1, 11; P > 0.05
Long s. trichodea (Tr-I)	$6.9 \times 10^{-3} \pm 3.4 \times 10^{-4}$**b**	$8.9 \times 10^{-3} \pm 4.1 \times 10^{-4}$**a**	$5.6 \times 10^{-3} \pm 4.5 \times 10^{-4}$**c**	$6.4 \times 10^{-3} \pm 2.2 \times 10^{-4}$**b**	F = 13.5; df = 1, 16; P < 0.01	F = 21.4; df = 1, 16; P < 0.001	F = 1.95; df = 1, 16; P > 0.05
Short s. trichodea (Tr-II)	$2.1 \times 10^{-3} \pm 2.7 \times 10^{-4}$	$1.7 \times 10^{-3} \pm 2.5 \times 10^{-4}$	$1.3 \times 10^{-3} \pm 2.5 \times 10^{-4}$	$1.7 \times 10^{-3} \pm 2.5 \times 10^{-4}$	F = 0.02; df = 1, 16; P > 0.05	F = 1.9; df = 1, 16; P > 0.05	F = 1.7; df = 1, 16; P > 0.05
Curved s. trichodea (Tr-III)	$2.4 \times 10^{-3} \pm 2.4 \times 10^{-4}$	$3.3 \times 10^{-3} \pm 3.1 \times 10^{-4}$			F = 4.01; df = 1, 9; P > 0.05		
Small s. basiconica (SB)	$2.8 \times 10^{-3} \pm 3.5 \times 10^{-4}$	$2.6 \times 10^{-3} \pm 5.8 \times 10^{-4}$			F = 0.14; df = 1, 9; P > 0.05		
Large s. basiconica (LB)	$4.0 \times 10^{-3} \pm 4.5 \times 10^{-4}$	$4.1 \times 10^{-3} \pm 6.7 \times 10^{-4}$			F = 0.01; df = 1, 9; P > 0.05		
Thin s. basiconica (TB)	$8.6 \times 10^{-4} \pm 5.2 \times 10^{-5}$	$1.1 \times 10^{-3} \pm 8.6 \times 10^{-4}$			F = 4.16; df = 1, 9; P > 0.05		
Grooved s. basiconica (GB)			$1.9 \times 10^{-2} \pm 1.5 \times 10^{-4}$	$9.5 \times 10^{-3} \pm 6.7 \times 10^{-4}$	F = 4.2; df = 1, 7; P > 0.05		
Long grooved s. basiconica (MpGB)	$1.7 \times 10^{-3} \pm 2.6 \times 10^{-4}$	$1.8 \times 10^{-3} \pm 4.1 \times 10^{-5}$	$2.4 \times 10^{-3} \pm 1.7 \times 10^{-4}$	$1.9 \times 10^{-3} \pm 8.9 \times 10^{-5}$	F = 0.5; df = 1, 14; P > 0.05	F = 3.2; df = 1, 14; P > 0.05	F = 2.1; df = 1, 14; P > 0.05
Grooved pegs (GP)	$8.7 \times 10^{-4} \pm 8.3 \times 10^{-5}$	$9.9 \times 10^{-4} \pm 1.4 \times 10^{-4}$	$1.1 \times 10^{-3} \pm 2.5 \times 10^{-4}$	$5.3 \times 10^{-4} \pm 1.6 \times 10^{-4}$	F = 0.95; df = 1, 14; P > 0.05	F = 0.06; df = 1, 14; P > 0.05	F = 4.7; df = 1, 14; P = 0.05

(Table 2). The LB, SB and TB subtypes are only found on the postpedicel of *M. signaticornis*, while the GB subtype is only found on the postpedicel of *M. infumata*.

In *M. signaticornis*, s. basiconica are situated within a shallow depression at the top part of the postpedicel (Fig. 1C). The LB is the most common sensillum subtype (Fig. 4G; Table 2) and is present in various forms, which not always end up in a rounded tip. The SB are mostly distributed around the LB sensilla, and are considerably longer than the LB but smaller than the TB (Fig. 4H; Table 2). Both SB and LB sensilla have a smooth cuticular surface with a high density of pores and a seemingly uniform distribution. The TB sensilla are less common on the postpedicel of *M. signaticornis*

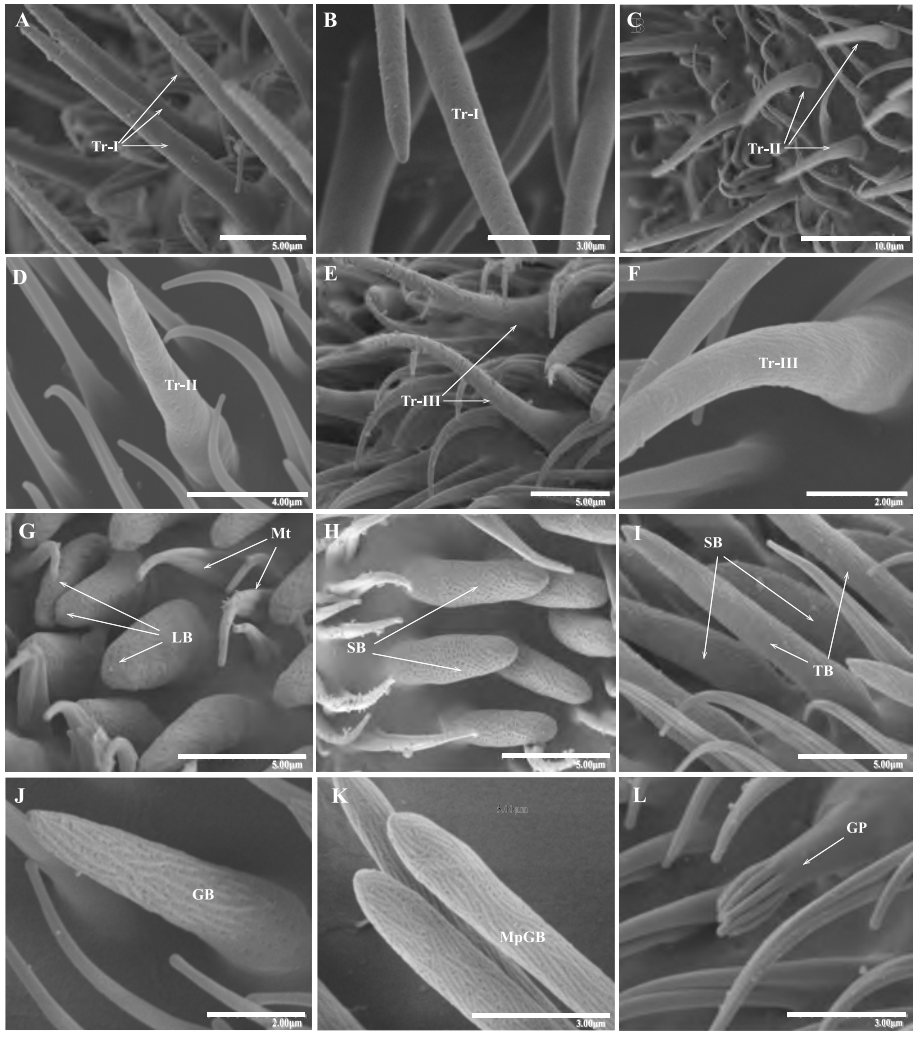

Fig. 4. High-resolution scanning micrographs showing the morphologically different olfactory sensillum types. **A-I** All pictures from the inner part of the postpedicel of female *M. signaticornis*. **A-B.** Long sensilla trichodea (Tr-I). **C-D.** Short s. trichodea (Tr-II). **E-F.** Curved s. trichodea (Tr-III). **G.** Large s. basiconica (LB) and microtrichia (Mt). **H.** Small s. basiconica (SB). **I.** Thin s. basiconica (TB) and SB. **J.** Grooved s. basiconica (GB) from the inner part of the postpedicel of a male *M. infumata*. **K.** Long grooved s. basiconica (MpGB) from the outer part of maxillary palp of a female *M. signaticornis*. **L.** Grooved peg sensillum (GP) from the postpedicel of a male *M. signaticornis*.

compared to the other two subtypes found in this species. This sensillum subtype is mainly located at the narrow edges of the shallow depression at the top medial part of the postpedicel. The TB sensilla were the longest s. basiconica subtype detected (Table 2). The TB sensilla have a sharp tip and a ridged cuticular surface with sparse pores (Fig. 4I).

The GB sensilla (Fig. 4J) are uniformly distributed on both the inner and outer sides of the postpedicel of *M. infumata* (Fig. 1D). Although the *M. infumata* GB and *M. signaticornis* TB sensilla found on the postpedicel are similar in length (Table 2), the cuticular surface of the GB sensilla contains more prominent ridges and a lower number of visible pores compared to the TB sensilla. Similar GB sensilla are also found grouped in a pit on the maxillary palps of both species (Fig. 3A, B, F). However, these sensilla are longer, blunt-tipped and are defined as MpGB (Fig. 4K; Table 2). No significant differences between densities MpGB on the maxillary palps are observed between species ($F = 3.2$; $df = 1, 14$; $P > 0.05$) or sexes ($F = 0.5$; $df = 1, 14$; $P > 0.05$), and the variables sex and species do not affect each other ($F = 2.1$; $df = 1, 14$ $P > 0.05$) (Table 3).

3.6. Grooved pegs

The GP are the smallest and least abundant sensillum type found in both species (Table 2). This sensillum type is located

mainly around the basal part of the postpedicel, close to pedicel. These sensilla are characterised by finger-like processes that extend from an otherwise smooth cuticular shaft (Fig. 4L). No significant differences in the density of GP are observed between species ($F = 0.06$; $df = 1, 14$; $P > 0.05$) or sexes ($F = 0.95$; $df = 1, 14$; $P > 0.05$), and no statistical interaction is seen between these two variables ($F = 4.7$; $df = 1, 14$ $P > 0.05$) (Table 3).

4. Discussion

Many *Medetera* species are predators on bark beetles, which they locate using olfaction. Through a comparative analysis of the main olfactory organs of *M. signaticornis* and *M. infumata*, this study demonstrates significant differences between species in relation to surface areas of the postpedicel and the maxillary palps, the subtypes of antennal sensilla (trichodea and basiconica) and the sensillum densities on the antennae and the maxillary palps. The olfactory system is known to reflect insect phylogeny, ecology and ethology (Hansson and Stensmyr, 2011) and the observed differences in morphology likely reflect evolutionary adaptations related to ecologically relevant behaviours, such as host finding and reproduction. In contrast, the shape and location of the different sensilla found on the maxillary palps are well conserved.

4.1. Antennal morphology

The antennae of *M. signaticornis* and *M. infumata* are composed of three distinct segments: the scape, the pedicel and the postpedicel with a terminal arista-like stylus, similar to the antennal morphology in most fly species (Diptera: Brachycera) (Cumming and Wood, 2017). The scape and the pedicel have been suggested to have a baroreceptor role in the majority of families of Diptera (McAlpine, 2011). Within the genus *Medetera*, the scape is usually relatively short and sometimes rudimentary, but some *Medetera* species can have a more elongated scape compared to the pedicel (Bickel, 1985). Within the Dolichopodidae, the pedicel can also be round and laterally compressed or featuring a finger-like inner projection (e.g., in species from the genus *Syntormon* Loew, 1857), which penetrates the inner side of the postpedicel (Grichanov, 2007). The external structure of the pedicel is taxonomically variable across different dipteran families (Cumming and Wood, 2017). In more distantly-related dipteran species, the pedicel can be enlarged (e.g., members of family Tabanidae), elongated (e.g., members of families Conopidae and Sciomyzidae) or it can be marked dorsally by a longitudinal "antennal seam" (e.g., members of families Muscidae and Calyptratae) (McAlpine et al., 1981; McAlpine, 2011). The functional relevance for the observed morphological variation in scape and pedicel is yet unknown. In all cases, the pedicel connects to the third antennal segment, the postpedicel.

As compared to other dolichopodids, both *M. signaticornis* and *M. infumata* have a relatively small postpedicel of oval shape. Previous studies have shown that shape and size of postpedicel varies across *Medetera* species from elongated triangular (e.g., *M. ambigua* Zetterstedt, 1843) to large triangular (e.g., *M. pinicola* Kowarz, 1878 and *M. melancholica* Lundbeck, 1912) or quadrate of various sizes (e.g., other species of the *M. signaticornis-pinicola* group) (Bickel, 1985). Postpedicel size and shape also differ substantially between genera within the same dolichopodid subfamilies (Negrobov et al., 2015). While not observed in our study, in other fly species representing several dolichopodid genera like e.g., *Argyra* Macquart, 1834; *Dolichopus* Latreille, 1796; *Rhaphium* Meigen, 1803; *Syntormon* and some *Sybistroma* Meigen, 1824, a sexual dimorphism can be found in the shape or size of the postpedicel (Pollet, 2000; Negrobov et al., 2015). Whether this dimorphism reflects a function in sexual communication remains to be determined.

The arista-like stylus has previously been described within the *Medetera* genus as long, bi-segmented and borne apically in a slight notch almost at the tip of the postpedicel (Bickel, 1985). The arista-like stylus is bi-segmented in all dolichopodids, varies strongly in length and can be positioned in various places along the dorsal side of the postpedicel (from basodorsal to apical). However, in some cases within the Dolichopodidae the second segment of the arista-like stylus can be extended (e.g., species of the genera *Chrysosoma* Guerin-Meneville, 1831 and *Plagiozopelma* Enderlein, 1912) (Negrobov et al., 2015). It can also be bare (e.g., species of the genus *Tachytrechus* Haliday, 1851), have short hairs (e.g., species of the genus *Medetera*) or be very pilose (e.g., species of the genus *Poecilobothrus* Mik, 1857) (Negrobov et al., 2015). Still, within the Dolichopodidae family, some species (e.g., genus *Sybistroma*) show apical and even median flags on the second segment of the arista-like stylus (Grichanov, 2007).

Similar arista-like stylus morphology, as observed in both of the studied *Medetera* species, has been described in members of other Diptera families, such as species of genera *Callomyia* Meigen, 1804 (Diptera: Platypezidae) (Cumming and Wheeler, 2016), *Macalpinomyia* Li and Yeates, 2018 (Diptera: Ironomyiidae) (Li and Yeates, 2019), *Megaselia* Rondani, 1856 (Diptera: Phoridae) and *Pseudacteon* Coquillett, 1907 (Diptera: Phoridae) (Pfeil et al., 1994; Sukontason et al., 2005; Chen and Fadamiro, 2008; Lu, 2012). However, other dipteran species can have a subterminal or terminal stylus, which usually is short (e.g., *Mallophora ruficauda* Wiedemann, 1828 (Diptera: Asilidae) (Groba et al., 2014) and *Rhamphomyia bhagati* sp. nov. (Diptera: Tabanidae) (Barták et al., 2021)). Alternatively, dipteran species can have a bristle-like arista, which originates from the inner or outer surface, near the lateral edge, of the postpedicel (e.g., *Gymnosoma rotundatum* Linnaeus, 1758 (Diptera: Tachinidae) (Roh et al., 2020)) or from the dorso-proximal end of the postpedicel (e.g., species of genus *Drosophila* Fallén, 1823 (Diptera: Drosophilidae) (Shanbhag et al., 1999; Gao et al., 2020)). In *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae), the arista has been demonstrated to play a role in both humidity (Sayeed and Benzer, 1996; Ji and Zhu, 2015) and auditory sensing (Yorozu et al., 2009). However, the function of the arista-like stylus of *Medetera* and other flies remains to be understood.

4.2. Antennal sensillar equipment

In both *Medetera* species, the sensilla of type Ch are found to be organized in a ring around the pedicel. This is similar to that described for most members of the Dolichopodidae family (Grichanov, 2007), with the exception of the *M. aberrans* Wheeler, 1899 group, which has a reduced number of this sensillum type (Bickel, 1985), likely rendering them less capable of detecting mechanosensation, i.e., the sensory modality conveyed by these sensilla (Shanbhag et al., 1999).

In comparison with other families of flies, the distribution of this sensillum type varies considerably. The distribution can be confined to only the scape or the pedicel (e.g., *G. rotundatum* (Roh et al., 2020)) or both the scape and the pedicel can have this sensillum type. Examples of the latter can be found in species such as *M. ruficauda* and *Eupeodes corollae* Fabricius, 1794 (Diptera: Syrphidae) and in species of the genera *Tabanus* Linnaeus, 1758 (Diptera: Tabanidae) and *Simulium* Latreille, 1802 (Diptera: Simuliidae) (Mercer and McIver, 1973; Parashar et al., 1994; Groba et al., 2014; Jia et al., 2019). In Simuliidae, the arrangement of sensilla type Ch around the distal boundary of the scape and pedicel suggests that they may detect the direction and degree of bending of the scape and pedicel, emphasizing a role in assessing air movement over the antenna (Mercer and McIver, 1973).

Sensilla trichodea are one of the most abundant sensillum type found in both *M. signaticornis* and *M. infumata*. This is similar to other fly species (e.g., species from genus *Pseudacteon* (Chen and Fadamiro, 2008; Lu, 2012), *E. corollae* (Jia et al., 2019) and *M. ruficauda* (Groba et al., 2014)). Similar to *Medetera*, the abundance of this sensillum type has been observed to increase from the base to the distal end of the postpedicel in species of genera *Pseudacteon*, *Megaselia* and *Apystomyia* Melander, 1950 (Diptera: Apystomyiidae) (Chen and Fadamiro, 2008; McAlpine, 2011; Lu, 2012). Based on their ultrastructure, s. trichodea have been attributed an olfactory function, and shown to respond to chemical signals used for e.g., foraging, oviposition and mate seeking by dipterans (De Bruyne et al., 2001; Qiu et al., 2006; Hill et al., 2009; Siju et al., 2010). Given the higher abundance of s. trichodea on the postpedicel in males of both studied *Medetera* species, it is a possibility that this sensillum type may be tuned to detect pheromones, as in *D. melanogaster* (Clyne et al., 1997), although pheromone compounds are yet to be identified in Dolichopodidae.

Sensilla basiconica are another sensillum type found with uniform distribution on the postpedicel of *M. infumata*, and are concentrated in a shallow depression at the top part on the postpedicel of *M. signaticornis*. Morphologically, similar subtypes of s. basiconica have been described in *Pseudacteon tricuspis* Borgmeier, 1925 (Diptera: Phoridae) (Chen and Fadamiro, 2008), *M. ruficauda* (Groba et al., 2014), and *G. rotundatum* (Roh et al., 2020). However, the density and distribution of this sensillum type in members of these and other families of flies differs from the two studied *Medetera* species (Shanbhag et al., 1999; Chen and Fadamiro, 2008; Groba et al., 2014; Roh et al., 2020). Functional analysis of s. basiconica in other species has demonstrated that this sensillum type responds to a variety of odorants, including those involved in the detection of food and oviposition sites (De Bruyne et al., 1999, 2001; Elmore et al., 2003). The differences we found in relation to the number and subtypes of s. basiconica between the two *Medetera* species, might reflect species-specific habitat preference. *M. signaticornis* is mainly known to be associated with coniferous forests where bark beetle damage occurs. In contrast, *M. infumata* belongs to a species group that is not confined to tree trunks but also occurs on dry sandy soils (Bickel, 1985; Pollet et al., 2011). Recently, *M. infumata* has been recorded from wooded habitats with coniferous trees, where it is found on both tree trunks and rocky substrates (Pollet, pers. comm.). Accordingly, in slight difference to our hypothesis, the olfactory organs and sensillar equipment show dissimilarities, which might reflect adaptations in relation to species-specific ecology and behaviours, despite common coexistence in coniferous forest, egg-laying on bark beetle-infested trees and attraction to bark beetle pheromones. Future functional analysis is required to assess a possible correlation between this sensillum type, specific chemosensory stimulants and behavioural preference.

The GP described in both *M. signaticornis* and *M. infumata* have previously been found in both members of closely and distantly-related families of dipteran flies, such as *Megaselia scalaris* Loew, 1866 (Diptera: Phoridae) (Sukontason et al., 2005), *P. tricuspis* (Chen and Fadamiro, 2008), *Trichopoda pennipes* Fabricius, 1781 (Diptera: Tachinidae) (Giangiuliani et al., 1994), *Haematopota pandazisi* Krober, 1936 (Diptera: Tabanidae) (Pezzi et al., 2018), *D. melanogaster* (Shanbhag et al., 1999), *Culicoides obsoletus* Meigen, 1818 (Diptera: Ceratopogonidae) (Isberg et al., 2013; Urbanek et al., 2014) and *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae) (Boo and McIver, 1976; Hempolchom et al., 2017). This type of sensillum is ancestral and seems to be conserved through hundreds of millions of years of insect evolution as it can be found in many, if not in all, insect orders (Steinbrecht, 1997; Yao et al., 2005). However, the distribution of this sensillum type on the antenna varies greatly across insect families. Most of the work on the GP has been done in *D. melanogaster* showing that it is tuned to carboxylic acids and amines, expresses olfactory receptors of the ionotropic receptor family but also plays a role in other sensory modalities (Meijerink et al., 2001; Yao et al., 2005; Ghaninia et al., 2008).

4.3. Maxillary palp

The maxillary palp of both *M. signaticornis* and *M. infumata* consists of one small, flat and oval segment. This seems to be similar for the majority of the species of *Medetera* genus (Bickel and Arnaud Jr, 2011). In other genera, within the Dolichopodidae family, the shape of maxillary palps varies substantially e.g., it can also be subquadrate with rounded corners (e.g., *Chrysotus* Meigen, 1824), or rather narrow and pointed apically (e.g., *Amblypsilopus* Bigot, 1888) (Grichanov, 2007). The size also varies from small (e.g., *Sympycnus* Loew, 1857) to large (e.g., *Tachytrechus*) (Brooks and Cumming, 2008) or extended (e.g., *Chrysotus*) (Capellari, 2015; Runyon and Capellari, 2018). Compared to other fly families, the morphology of the maxillary palp varies in relation to the number of palpal segments, orientation (e.g., arched, straight, curved or C-shaped), length and attachment (e.g., connected directly to stipes, separated from stipes, connected to an external sclerite or palpifer) (Sinclair and Cumming, 2006). Additionally, although not observed in our study, sexual dimorphism can be found in relation to the length and shape of the maxillary palps (Runyon, 2020; Pezzi et al., 2021).

4.4. Palpal sensillar equipment

On the maxillary palps of both *Medetera* species, two subtypes of sensilla Ch were found, differentiated based on their size and position. The small Ch-I were distributed from the centre to the distal end of the maxillary palp, while only one long Ch-II was found in the medial part of the palp. In the descriptions of *Medetera* species, the maxillary palp has been mentioned as bearing apical small hairs on the outer side and one long bristle at the apex (Bickel, 1985; Grichanov, 2007). Apical hairs, and one or several long bristles, are also common in distantly-related species such as *D. melanogaster* (Shanbhag et al., 1999) and *Bactrocera dorsalis* Hendel, 1912 (Diptera: Tephritidae) (Liu et al., 2021).

In addition to Ch-I and II, the maxillary palps of both *Medetera* species carry the sensilla MpGB grouped in a pit. Similar sensory pits can be found in closely-related families of predatory and parasitoid flies (e.g., *Hilara maura* Fabricius, 1776 (Diptera: Empididae), *Stenopogon inquinatus* Loew, 1866 (Diptera: Asilidae) and *Lomatia belzebul* Fabricius, 1794 (Diptera: Bombyliidae) (Yeates, 1994; Sinclair and Cumming, 2006), and in distantly-related flies (e.g., species within the genus *Culicoides* Latreille, 1809 (Diptera: Ceratopogonidae) (Isberg et al., 2013). Single sensillum recordings of the papal s. basiconica in different mosquito species (e.g., species within the genera *Anopheles* Meigen, 1818; *Culex* Linnaeus, 1758 and *Culicoides*) have shown that this sensillum type detects carbon dioxide (CO_2), which is a generic cue shared by all vertebrate hosts (Grant and Kline, 2003; Grant et al., 1995). Similarly, CO_2 might be a cue used for example by predatory flies to detect small soft-bodied arthropods that they feed on.

We hypothesized similarity in the morphology of the olfactory organs and sensillar equipment between *M. signaticornis* and *M. infumata,* as based on their close phylogenetic relation and overlap in their ecology and behaviour. In conclusion of our study, we found that the general morphology of the antennae and maxillary palps, in relation to the shape, size and position is similar between *M. signaticornis* and *M. infumata*, but the antennal sensillar equipment varies across species. Different sensillum subtypes and

distributions might indicate that *M. signaticornis* and *M. infumata* process different environmental cues or process the same cues in a different way. However, future studies need to be done to gain a better understanding of the diversity, physiological function and evolution of sensilla types within this taxonomic group. Investigations of the sensorial structures in the *Medetera* genus may establish a base for studies that aim at understanding how these predators find their hosts, mates and oviposition sites. Better understanding of the host finding behaviour might advance the development of biological control of bark beetles with *Medetera* flies.

Author contributions

MS, **PGB**, **KKG** and **GB** planned and conceived the study. **MS** Collected, analysed the data and wrote the original draft of the manuscript. **RI** assisted with the identification of the different olfactory sensillum types, structure and revision of the manuscript. **MP** identified the specimens and gave guidance during the early stage of the study. **GB** initiated the project. All co-authors contributed to the revision of the manuscript and have approved the last version of the manuscript.

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References

Barták, M., Akbar, S.A., Kanturski, M., Wackhoo, A.A., Maqbool, A., 2021. SEM morphology and courtship rituals of a new species of *Rhamphomyia* (Diptera: Empididae: Empidinae) from the Kashmir Himalayas (India). Bonn Zool. Bull. 70, 67–84.

Beaver, R., 1966. The biology and immature stages of two species of *Medetera* (Diptera: Dolichopodidae) associated with the bark beetle *Scolytus scolytus* (F.). Proc. R. Ent. Soc. Lond. 41, 145–154.

Bickel, D.J., 1985. A Revision of the Nearctic *Medetera* (Diptera: Dolichopodidae), United States Department of Agriculture, Agricultural Research Service. Technical Bulletin Number 1692.

Bickel, D.J., Arnaud JR., P.H., 2011. *Medetera johnthomasi* (Diptera: Dolichopodidae), a new species from California with notes on the *aberrans* species group. Pan-Pacific Entomol. 87, 124–129.

Boo, K.S., McIver, S., 1976. Fine structure of surface and sunken grooved pegs on the antenna of female *Anopheles stephensi* (Diptera: Culicidae). Can. J. Zool. 54, 235–244.

Brooks, S.E., Cumming, J.M., 2008. The *Tachytrechus alatus* species group (= *Syntomoneurum* Becker) revisited: new species and revised species group limits (Diptera: Dolichopodidae). Zootaxa 1676, 1–27.

Capellari, R.S., 2015. Df Review of the *longipalpus*-group of *Chrysotus* Meigen (Diptera: Dolichopodidae), with description of four new species. Neotrop. Entomol. 44, 47–58.

Chen, L., Fadamiro, H.Y., 2008. Antennal sensilla of the decapitating phorid fly, *Pseudacteon tricuspis* (Diptera: Phoridae). Micron 39, 517–525.

Clyne, P., Grant, A., O'Connell, R., Carlson, J.R., 1997. Odorant response of individual sensilla on the *Drosophila* antenna. Invertebr. Neurosci. 3, 127–135.

Cumming, H.J., Wheeler, T.A., 2016. Revision of the nearctic species of *Callomyia* meigen (Diptera: Platypezidae) and phylogeny of the genus. Zootaxa 4111, 501–554.

Cumming, J., Wood, D., 2017. Adult morphology and terminology [chapter 3]. In: Kirk-Spriggs, A.H., Sinclair, B.J. (Eds.), Manual of Afrotropical Diptera, Suricata 4, vol. 1. SANBI Publ., Pretoria, pp. 89–133.

De Bruyne, M., Clyne, P.J., Carlson, J.R., 1999. Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. J. Neurosci. 19, 4520–4532.

De Bruyne, M., Foster, K., Carlson, J.R., 2001. Odor coding in the *Drosophila* antenna. Neuron 30, 537–552.

De Leon, D., 1935. A study of *Medetera aldrichii* Wh. (Diptera—Dolichopodidae), a predator of the mountain pine beetle (*Dendroctonus monticolae* Hopk., Coleo.—scolytidae). Entomol. Am. 15, 59–91.

Dippel, C., Heidger, C., Nicolai, V., Simon, M., 1997. The influence of four different predators on bark beetles in European forest ecosystems (Coleoptera: Scolytidae). Entomol. Gen. 21, 161–175.

Elmore, T., Ignell, R., Carlson, J.R., Smith, D.P., 2003. Targeted mutation of a *Drosophila* odor receptor defines receptor requirement in a novel class of sensillum. J. Neurosci. Res. 23, 9906–9912.

Gao, H., Lai, S., Zhai, Y., Lv, Z., Zheng, L., Yu, Y., Ren, F.S., 2020. Comparison of the antennal sensilla and compound eye sensilla in four *Drosophila* (Diptera: Drosophilidae) species. Fla. Entomology (Tokyo) 102, 747–754.

Ghaninia, M., Larsson, M., Hansson, B.S., Ignell, R., 2008. Natural odor ligands for olfactory receptor neurons of the female mosquito *Aedes aegypti*: use of gas chromatography-linked single sensillum recordings. J. Exp. Biol. 211, 3020–3027.

Giangiuliani, G., Lucchi, A., Vinson, S.B., Bin, F., 1994. External anatomy of adult antennal sensilla of the fly, *Trichopoda pennipes* F. (Diptera: Tachinidae). Insect Morphol. Embryol. 23, 105–113.

Grant, A., Aghajanian, J., O'Connel, R., Wigton, B., 1995. Electrophysiological responses of receptor neurons in mosquito maxillary palp sensilla to carbon dioxide. J. Comp. Physiol. A. 177, 389–396.

Grant, A.J., Kline, D.L., 2003. Electrophysiological responses from *Culicoides* (Diptera: Ceratopogonidae) to stimulation with carbon dioxide. J. Med. Entomol. 40, 284–292.

Grichanov, I.Y., 2007. A Checklist and Keys to Dolichopodidae (Diptera) of the Caucasus and East Mediterranean. Plant Protection News Supplements, St. Petersburg: VIZR,, pp. 1–160.

Groba, H.F., De Cidre, L.L., Castelo, M.K., 2014. Description of antennal structures of the parasitoid *Mallophora ruficauda* (Diptera: Asilidae) and its relationship with resources searching behaviour. Zoomorphology 133, 191–204.

Hallberg, E., Hansson, B.S., 1999. Arthropod sensilla: morphology and phylogenetic considerations. Microsc. Res. Tech. 47, 428–439.

Hansson, B.S., Stensmyr, M.C., 2011. Evolution of insect olfaction. Neuron 72, 698–711.

Hedgren, P.O., Schroeder, L.M., 2004. Reproductive success of the spruce bark beetle *Ips typographus* (L.) and occurrence of associated species: a comparison between standing beetle-killed trees and cut trees. For. Ecol. Manage 203, 241–250.

Hempolchom, C., Yasanga, T., Wijit, A., Taai, K., Dedkhad, W., Srisuka, W., Thongsahuan, S., Otsuka, Y., Takaoka, H., Saeung, A., 2017. Scanning electron microscopy of antennal sensilla of the eight *Anopheles* species of the Hyrcanus Group (Diptera: Culicidae) in Thailand. Parasitol. Res. 116, 143–153.

Hill, S.R., Hansson, B.S., Ignell, R., 2009. Characterization of antennal trichoid sensilla from female southern house mosquito, *Culex quinquefasciatus* Say. Chem. Senses 34, 231–252.

Hulcr, J., Pollet, M., Ubik, K., Vrkoc, J., 2005. Exploitation of kairomones and synomones by *Medetera* spp. (Diptera: Dolichopodidae), predators of spruce bark beetles. Eur. J. Entomol. 102, 655–662.

Hulcr, J., Ubik, K., Vrkoc, J., 2006. The role of semiochemicals in tritrophic interactions between the spruce bark beetle *Ips typographus*, its predators and infested spruce. J. Appl. Entomol. 130, 275–283.

Isberg, E., Hillbur, Y., Ignell, R., 2013. Comparative study of antennal and maxillary palp olfactory sensilla of female biting midges (Diptera: Ceratopogonidae: Culicoides) in the context of host preference and phylogeny. J. Med. Entomol. 50, 485–492.

Ji, F., Zhu, Y., 2015. A novel assay reveals hygrotactic behavior in *Drosophila*. PLoS One 10, e0119162.

Jia, H.-R., Sun, Y.-F., Luo, S.-P., Wu, K.-M., 2019. Characterization of antennal chemosensilla and associated odorant binding as well as chemosensory proteins in the *Eupeodes corollae* (Diptera: Syrphidae). J. Insect Physiol. 113, 49–58.

Keil, T.A., 1999. Morphology and development of the peripheral olfactory organs. In: Hansson, B.S. (Ed.), Insect Olfaction. Springer, Berlin, Heidelberg, pp. 5–47.

Li, X., Yeates, D.K., 2019. The first Ironomyiidae from mid-Cretaceous Burmese amber provides insights into the phylogeny of Phoroidea (Diptera: Cyclorrhapha). Syst. Entomol. 44, 251–261.

Lin, C.-C., Potter, C.J., 2015. Re-classification of *Drosophila melanogaster* trichoid and intermediate sensilla using fluorescence-guided single sensillum recording. PLoS One 10, e0139675.

Liu, Z., Hu, T., Guo, H.W., Liang, X.F., Cheng, Y.Q., 2021. Ultrastructure of the olfactory sensilla across the antennae and maxillary palps of *Bactrocera dorsalis* (Diptera: Tephritidae). Insects 12, 289.

Lu, Y., 2012. Types of antennal sensilla of three *Pseudacteon* species (Diptera: Phoridae) females that parasitize red imported fire ants (*Solenopsis invicta*) (Hymenoptera: Formicidae). Sociobiology 59, 1535–1546.

McAlpine, D.K., 2011. Observations on antennal morphology in Diptera, with particular reference to the articular surfaces between segments 2 and 3 in the Cyclorrhapha. Record Aust. Mus. 63, 113–166.

McAlpine, J.F., Peterson, B., Shewell, G., Teskey, H., Vockeroth, J., Wood, D., 1981. Manual of Nearctic Diptera, vol. 1. Agriculture Canada.

Meijerink, J., Braks, M., Van Loon, J., 2001. Olfactory receptors on the antennae of the malaria mosquito *Anopheles gambiae* are sensitive to ammonia and other sweat-borne components. J. Insect Physiol. 47, 455–464.

Mercer, K.L., Mciver, S.B., 1973. Studies on the antennal sensilla of selected blackflies (Diptera: Simuliidae). Can. J. Zool. 51, 729–734.

Negrobov, O.P., Chursine, M.A., Selivanova, O.V., 2015. Antennal morphology in the family Dolichopodidae (Diptera). J. Insect Biodivers. 3, 1–10.

Nuorteva, M., 1956. Über den Fichtenstamm-Bastkäfer, *Hylurgops palliatus* Gyll., und seine Insektenfeinde. Acta Entomol. Fenn. 13, 7–116.

Parashar, B.D., Chauhan, R.S., Prakash, S., Rao, K.M., 1994. Mechanotactile and olfactory antennal sensilla in four species of female tabanids (Diptera). Ital. J. Zool.

61, 121–128.
Pezzi, M., Scapoli, C., Bharti, M., Faucheux, M., Chicca, M., Leis, M., Marchetti, M., Mamolini, E., Salvia, R., Falabella, P., 2021. Fine structure of maxillary palps in adults of Hermetia illucens (Diptera: Stratiomyidae). J. Med. Entomol. 58, 658–665.
Pezzi, M., Scapoli, C., Mamolini, E., Leis, M., Bonacci, T., Whitmore, D., Krcmar, S., Furini, M., Giannerini, S., Chicca, M., 2018. Ultrastructural characterization of sensilla and microtrichia on the antenna of female *Haematopota pandazisi* (Diptera: Tabanidae). Parasitol. Res. 117, 959–970.
Pfeil, R., Walsh, R., Mumma, R., 1994. Scanning electron microscopic examination of the putative olfactory structures possessed by the phorid fly, *Megaselia halterata* (Diptera, Phoridae). Scanning Microsc. 8, 25.
Pitts, R.J., Zwiebel, L.J., 2006. Antennal sensilla of two female anopheline sibling species with differing host ranges. Malar. J. 5, 1–12.
Pollet, M., 2000. A documented red list of the dolichopodid flies (Diptera: Dolichopodidae) of Flanders. Commun. Inst. Nat. Conserv. 8, 1–190.
Pollet, M., 2011. Fauna Europaea: Dolichopodidae. Fauna Europaea: Diptera, Brachycera. Fauna Europaea v2. http://www.faunaeur.org.
Pollet, M., Germann, C., Bernasconi, M.V., 2011. Phylogenetic analyses using molecular markers reveal ecological lineages in *Medetera* (Diptera: Dolichopodidae). Can. Entomol. 143, 662–673.
Qiu, Y.T., Van Loon, J.J., Takken, W., Meijerink, J., Smid, H.M., 2006. Olfactory coding in antennal neurons of the malaria mosquito, *Anopheles gambiae*. Chem. Senses 31, 845–863.
Roh, G.H., Lee, Y.J., Park, C.G., 2020. Morphology and distribution of antennal sensilla in a parasitoid fly, *Gymnosoma rotundatum* (Diptera: Tachinidae). Microsc. Res. Tech. 83, 589–596.
Runyon, J.B., 2020. The Dolichopodidae (Diptera) of Montserrat, west Indies. ZooKeys 966, 57.
Runyon, J.B., Capellari, R.S., 2018. Palpi aplenty: new species in the *Chrysotus longipalpus* species group (Diptera: Dolichopodidae). Zootaxa 4399, 579–585.
Sayeed, O., Benzer, S., 1996. Behavioral genetics of thermosensation and hygrosensation in *Drosophila*. Proc. Natl. Acad. Sci. 93, 6079–6084.
Schneider, D., 1964. Insect antennae. Annu. Rev. Entomol. 9, 103–122.
Shanbhag, S., Müller, B., Steinbrecht, R., 1999. Atlas of olfactory organs of *Drosophila melanogaster*: 1. Types, external organization, innervation and distribution of olfactory sensilla. Int. J. Insect Morphol. Embryol. 28, 377–397.
Siju, K., Hill, S.R., Hansson, B.S., Ignell, R., 2010. Influence of blood meal on the responsiveness of olfactory receptor neurons in antennal sensilla trichodea of the yellow fever mosquito, *Aedes aegypti*. J. Insect Physiol. 56, 659–665.
Sinclair, B.J., Cumming, J.M., 2006. The morphology, higher-level phylogeny and classification of the Empidoidea (Diptera). Zootaxa 1180, 1–172.
Steinbrecht, R.A., 1997. Pore structures in insect olfactory sensilla: a review of data and concepts. Int. J. Insect Morphol. Embryol. 26, 229–245.
Sukontason, K., Sukontason, K.L., Vogtsberger, R.C., Boonchu, N., Chaiwong, T., Piangjai, S., Disney, H., 2005. Ultrastructure of coeloconic sensilla on postpedicel and maxillary palp of *Megaselia scalaris* (Diptera: Phoridae). Ann. Entomol. Soc. Am. 98, 113–118.
Urbanek, A., Piotrowicz, M., Szadziewski, R., Gilka, W., 2014. Sensilla coeloconica ringed by microtrichia in host-seeking biting midges. Med. Vet. Entomol. 28, 355–363.
Wegensteiner, R., Wermelinger, B., Herrmann, M., 2015. Natural Enemies of Bark Beetles: Predators, Parasitoids, Pathogens, and Nematodes. Bark Beetles. Academic Press, pp. 247–304.
Wermelinger, B., 2002. Development and distribution of predators and parasitoids during two consecutive years of an *Ips typographus* (Col., Scolytidae) infestation. J. Appl. Entomol. 126, 521–527.
Yang, D., Zhu, Y., Wang, M., Zhang, L., 2006. World Catalogue of Dolichopodidae (Insecta: Diptera). China Agric. Univ. Beijing, pp. 1–704.
Yao, C.A., Ignell, R., Carlson, J.R., 2005. Chemosensory coding by neurons in the coeloconic sensilla of the *Drosophila* antenna. J. Neurosci. Res. 25, 8359–8367.
Yeates, D.K., 1994. The cladistics and classification of the Bombyliidae (Diptera: Asiloidea). Bull. Am. Mus. Nat. Hist. 219, 1–19.
Yorozu, S., Wong, A., Fisher, B.J., Dankert, H., Kernan, M.J., Kamikouchi, A., Ito, K., Anderson, D.J., 2009. Distinct sensory representations of wind and near-field sound in the *Drosophila* brain. Nature 458, 201–205.
Zhang, D., Li, X., Liu, X., Wang, Q., Pape, T., 2016. The antenna of horse stomach bot flies: morphology and phylogenetic implications (Oestridae, Gasterophilinae: *Gasterophilus* Leach). Sci. Rep. 6, 1–20.

III

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Odors Attracting the Long-Legged Predator *Medetera signaticornis* Loew to *Ips typographus* L. Infested Norway Spruce Trees

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Abstract
Predatory long-legged flies of the genus *Medetera* are important, but currently understudied, natural enemies of Scolytinae bark beetles such as *Ips typographus*. *Medetera* flies lay eggs on beetle-infested trees, where the developing larvae find their prey, but the chemical cues used by *Medetera* to locate infested trees are currently unknown. To identify odors attracting *Medetera signaticornis,* a species in Europe, headspace samples were collected at several time-points through different stages of *I. typographus* attacks on logs of Norway spruce (*Picea abies*). The headspace samples were analyzed using combined gas chromatography and mass spectrometry (GC–MS), and gas chromatography coupled with electroantennographic detection (GC-EAD) to determine compounds that stimulate *M. signaticornis* antennae. Antennae of *M. signaticornis* males and females were found to detect (−)-*cis*-verbenol, (+)-*trans*-verbenol and myrtenol, which are known to be produced by bark beetles. Antennal responses were also observed for verbenene, isoterpinolene, α-pinene oxide, camphor, pinocamphone, terpinene-4-ol, myrtenal, borneol, α-terpineol, geranyl acetone, and verbenone, which are primarily produced by microorganisms, and α-pinene, α-fenchene, β-pinene, camphene, 3-carene, limonene, γ-terpinene, and terpinolene, known spruce tree compounds. In field experiments testing two synthetic blends containing 18 antennal active and two additional compounds 2-methyl-3-buten-2-ol and ipsdienol we observed significant attraction of *M. signaticornis* within 24 h. These attractive blends can form the basis for development of *Medetera* monitoring lures for use in future forest and pest management.

Keywords Biocontrol · Electrophysiology · Host location · Kairomone · Predator–prey interaction · Sustainable forestry

Introduction

Norway spruce (*Picea abies* (L.) Karst.), an ecologically and economically important conifer species, is increasingly being threatened by fungal disease (e.g., root and butt rot disease caused by *Heterobasidion* spp.) (Gunulf et al. 2013; Gomez-Gallego et al. 2022) and insect pests (Hannerz et al. 2002; Romashkin et al. 2020; Hlásny et al. 2021). One of the most severe pests on Norway spruce is the Eurasian eight-spined spruce bark beetle (*Ips typographus* Linnaeus) (Coleoptera: Curculionidae, Scolytinae) (Grégoire et al. 2015). Within the coniferous forest ecosystem, *I. typographus* feeds on dead or dying spruce trees and contributes to recycling of nutrients (Edmonds and Eglitis 1989). However, weather conditions such as strong wind, warm temperature and low rainfall can cause mass development of *I. typographus* in windthrown or draught-stressed spruce trees, and in consequence of a high beetle abundance even healthy trees get attacked (Rouault et al. 2006; Kärvemo and Schroeder 2010; Stadelmann et al. 2014). When colonizing living

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spruce trees, *I. typographus* uses an aggregation pheromone that facilitates mass attack (Birgersson et al. 1984; Schlyter et al. 1987) and introduces a blue-staining symbiotic fungus that metabolizes tree defense compounds toxic to the beetle (Hammerbacher et al. 2013; Wadke et al. 2016; Zhao et al. 2019). In addition, *I. typographus* performs better in warm temperatures, and physiological models predict more frequent outbreaks in European *Picea* forests due to climate change (Marini et al. 2017; Bentz et al. 2019). There is therefore a pressing need to find efficient and sustainable methods to control bark beetles.

Understanding chemo-ecological aspects underlying host finding and infestation of trees by the beetles as well as subsequent trophic interactions might facilitate the development of pest management methods by use of semiochemicals or natural enemies. Tree volatiles serve as signals in host recognition of bark beetles (Seybold et al. 2006). In coniferous trees, terpenoid compounds (primarily volatile mono- and sesquiterpenes or less-volatile diterpenes) are characteristic constitutive or inducible defense chemicals, respectively (Keeling and Bohlmann 2006; Martin et al. 2003). The emission of these compounds can increase as response to biotic and abiotic stress such as the attack by wood boring insects, high temperature or mechanical damage (Ghimire et al. 2016; Juráň et al. 2017; Holopainen et al. 2018).

Several natural enemies of bark beetle, such as parasitoids and predators, are attracted to bark beetle-infested trees by volatile chemical cues that originate from the host tree (e.g., mono- and sesquiterpenes), from bark beetles themselves (e.g., *I. typographus* produced oxygenated hemi- and monoterpene alcohols), and/or from associated microorganisms which the beetles transfer to the host trees and their offspring developing inside the galleries (e.g., oxygenated monoterpenes) (Leufvén et al. 1984; Leufvén and Birgersson 1987; Pettersson 2000; Kandasamy et al. 2016, 2021). Natural enemies can significantly reduce bark beetle populations and are considered to be environmentally safe and sustainable control agents (Wermelinger 2002; Kenis et al. 2007; Wegensteiner et al. 2015).

An important group of predator species of bark beetles are flies of the genus *Medetera* (Diptera: Dolichopodidae). At present these are not actively applied or considered as biocontrol agents in forest management. *Medetera* adults feed on a wide array of small invertebrates (Ulrich 2004), but the larvae of most tree trunk-dwelling *Medetera* species depend on bark beetles for their development. The adult long-legged flies can be observed on tree trunks from early spring throughout the whole summer and up to the first frost. *Medetera* females have been observed to oviposit in newly infested trunks shortly after infestation by bark beetles (Nicolai 1995; Wermelinger 2004). On infested trees, females inspect the bark surface with their ovipositor and lay their eggs near the entrance of bark beetle galleries. A few days later, the newly emerged larvae migrate into the galleries and start feeding on beetle eggs and larvae, and on pupae or newly emerged, callow bark beetle adults that are still concealed in the galleries and pupal chambers (Beaver 1966; Bickel 1985). To locate bark beetle-infested trees, the adult flies use volatile chemical cues (Hulcr et al. 2005, 2006). Information on the specific compounds required for host detection is scarce, but it is known that some *Medetera* spp., such as *M. setiventris* Thuneberg and *M. melancholica* Lundbeck, are attracted to the *I. typographus* aggregation pheromone, which consists of a mixture of (-)-*cis*-verbenol and 2-methyl-3-buten-2-ol (Hulcr et al. 2005, 2006). According to Hulcr et al. (2006), the number of *M. setiventris* attracted to traps increases when *I. typographus* aggregation pheromone is combined with ipsdienol. Furthermore, *M. signaticornis* Loew has been shown to be attracted to a mixture of host tree compounds such as α-pinene, β-pinene, camphene, and limonene dissolved in ethanol (Rudinsky et al. 1971), while α-pinene has been shown to stimulate oviposition in females of *M. aldrichii* Wheeler and seems to guide newly emerged larvae to prey gallery entrances (Fitzgerald and Nagel 1972).

Medetera signaticornis is described as one of the most common *I. typographus* predators in Europe (Ounap 2001; Wermelinger 2002), which makes it a good candidate species for use in a future biocontrol strategy. In this study, we tested the hypothesis that *M. signaticornis* adult flies use multiple semiochemicals to detect bark beetle-infested Norway spruce trees. In order to identify key compounds that attract *M. signaticornis* to infested spruce, we: *i)* compared the volatilome of bark beetle-infested standing trees, bark beetle-infested cut trees and non-infested spruce trees over time; *ii)* identified odor compounds from infested trees eliciting electroantennographic responses on the antennae of *M. signaticornis* adults; and *iii*) tested the effectiveness of synthetic olfactory-active compound blends under field conditions.

Material and Methods

Insects Males and females of *M. signaticornis* were collected with a mouth aspirator from bark beetle-infested spruce trees (*Picea abies*) at two different sites (1 and 2, 57.150°N, 14.765°E and 57.127°N, 14.780°E, respectively) close to the SLU field research station in Asa, Småland province, Sweden, between May and August during 2018 and 2019. The average of the daily maximum temperature between May and August 2018 and 2019 at Asa was 24.3 ± 4.5 °C and 20.6 ± 3.5 °C, respectively, while the average daily precipitation during the same period was 1.65 ± 4.6 mm and 3.55 ± 8.1 mm (more detailed weather data can be accessed from the Asa weather station, Anon

(2023)). Collected flies were placed individually in glass vials with humidified filter paper and transported to the laboratory, where they were kept starved at 4–8 °C until electrophysiology studies, which were carried out within a week collection of the flies.

Volatile Collection Odor samples for chemical and electrophysiology analysis were collected from non-infested standing trees, infested standing trees, and infested cut trees at the two sites during 2018. A total of 11 healthy mature standing spruce trees, approximately 40–60 years age, were randomly selected at the two sites. Four of these trees were cut with a chainsaw (three at site 1 and one at site 2), and the fallen trunks and two standing trees at each site were baited with synthetic *I. typographus* pheromones (see below) to induce controlled *I. typographus* attacks. The remaining three trees (one from site 1 and two from site 2) were left without pheromone bait and used as controls.

The bait used to attract bark beetles to both cut and standing trees consisted of single dispensers containing the synthetic *I. typographus* aggregation pheromone (Pheroprax®, BASF, Limburgerhof, Germany). The dispensers were suspended at 3 m height (measured from the bottom of the tree) on the bark of the standing trees, and cut trees. Once *I. typographus* beetles had started excavating galleries in the baited trees, the dispensers containing synthetic bark beetle pheromones were removed and headspace collection was started.

A curved aluminum grid (area 32 cm × 32 cm, with 1.5 cm distance between grid and bark) was attached to each tree at specific collection points between 1.5 and 2 m tree height (measured from the bottom of the tree), to provide an open space for volatile release (Fig. 1A-C). For the eight infested cut or standing trees, the aluminum grid was affixed to cover the entrance hole(s) of one or two bark beetle galleries. To collect the volatiles emanating from the bark surface, the aluminum grid was covered with a polyester roasting bag (Toppits®, Cofresco Frischhalteprodukte GmbH, Minden, Germany) wrapped around the tree bark, giving an open bark surface area for volatile release of ~ 9 dm^2. Nylon wire was used to tie the upper and lower edges of the polyester bag to the tree (Fig. 1B). The released volatiles were collected through an adsorbent column (3 × 55 mm PTFE Teflon® tube, inner diameter 3.0 mm, outer diameter 4.0 mm, filled with ~ 30 mg of Porapak Q (mesh 50/80, Waters, Milford, MA, USA)) that had been placed in the open space under the grid before wrapping with the polyester bag. The absorbent column was connected by silicone tubing to a battery-driven membrane pump (KNF NMP830KNDC, KNF, Sursee, Switzerland) and the air drawn through each column was adjusted to a flow rate of 150 $mL \cdot min^{-1}$ for 3 h. An additional Porapak Q column was connected to the pump for sampling potential contamination from the air outside the enclosed aluminum grid. Compared to the samples from the bark inside the grid, amounts of compounds trapped outside the grid were neglectable (data not shown). After headspace collection, the polyester bag was cut open and the column was transferred to a clean glass vial. All vials were sealed and transported to the laboratory in a container with ice. In the laboratory, each column was eluted with 500 µL of pentane (*puriss p.a*, Sigma-Aldrich, Saint Louis, MO, USA) and the eluate was stored at -20 °C. Headspace collections from the same collection points on the same experimental

Fig. 1 Setup used for headspace collections from logs of Norway spruce trees (*Picea abies*) through the different stages of *Ips typographus* attack. **A**) three sampled standing trees in one of the field sites; **B**) metal grid covered with a polyester roasting bag that was wrapped around the tree bark forming an enclosure for odor collections; **C**) material used: 1. battery; 2. sucking air pump; 3. adsorbent columns (3 × 55 mm); 4. air splitter; 5. Silicone tubing; 6. Cables to connect the battery to the air pump; 7. aluminum grid (area 32 × 32 cm); 8. polyester roasting bag

trees (C1-C7) were made approximately every 10–15 days over a period of two months (from 10th May for site 1 or 25th May for site 2 up to 25th July), until the new generation of bark beetles began to emerge. The starting time of headspace collections between sites differed slightly because bark beetles were active earlier at site 1 compared to site 2. However, collections C1 to C6 from sites 1 and 2 correspond to similar stages of bark beetle attack. Details about collection dates and climatic conditions can be found in the Supplementary Table 1. In total, we were able to collect and analyze 12 samples from non-infested Norway spruce trees, 26 samples from infested standing trees, and 25 samples from infested cut trees.

Analysis by Gas Chromatography Coupled to Mass Spectrometry (GC–MS) All odor samples were concentrated to 100–150 µL and combined with 10 µL of heptyl acetate (100 ng μL^{-1}) as internal standard. Then 2 µL of each sample were injected by auto-injector (G4567A) into a gas chromatograph (7890B, GC) with mass spectrometry detection (5977A MS) (all Agilent Technologies, Santa Clara, CA, USA). The GC was equipped with a 60 m × 0.25 mm fused silica column coated with DB-Wax (polyethylene glycol, df = 0.25 µm, Agilent Technologies). Helium was used as the mobile phase, with a constant flow rate of 35 $cm \cdot s^{-1}$. The temperature program increased from 40 °C (3 min hold) at 8 $°C \cdot min^{-1}$ to 225 °C, which was held for 10 min. Electron impact mass spectra were obtained at 70 eV. All compounds were tentatively identified by comparison of the mass spectra obtained against: *i*) reference mass spectra from our custom library (based on spectra of standards analyzed on our GC–MS devices), supplemented with commercially available MS libraries (NIST, Wiley), and *ii*) Kováts retention index (RI) with reference to public RI libraries (PheroBase) (El-Sayed 2016).

Insect Preparation and Analysis by Gas Chromatography Coupled to Electroantennographic Detection (GC-EAD) To identify odor compounds from infested trees eliciting electroantennographic responses on antennae of *M. signaticornis* adults, the flies were gently inserted into a disposable plastic pipette tip with the narrow opening cut wider to let the fly's head pass through while retaining the thorax and abdomen inside the tip. A piece of glass wool was stuffed into the tip behind the insect body to immobilize the fly. Two glass electrodes were filled with Beadle-Ephrussi Ringer solution and one was inserted into one of the fly's eyes (indifferent electrode), while the other electrode was connected to a fly's antenna and mounted on a 10 × preamplifier probe (Ockenfels Syntech GmbH, Buchenbach, Germany) attached to an Intelligent Data Acquisition Controller (IDAC-2, Ockenfels Syntech).

For each GC-EAD analysis, 2 µL of odor sample with internal standard were used (five replicates per fly sex). The GC column and temperature program applied were similar to those used for GC–MS analysis. Hydrogen was used as a mobile phase, at 45 $cm \cdot s^{-1}$. At the GC effluent, 4 psi of nitrogen was added and split 1:1 in a Gerstel 3D/2 low dead volume four-way cross (Gerstel GmbH & Co KG, Mülheim, Germany) for simultaneous flame ionization detection and EAD recording of the separated compounds. The compounds eluting from the effluent capillary for EAD were mixed with charcoal-filtered humidified air (1.5 $L \cdot min^{-1}$) in a glass tube (length 10 cm, inner diameter 6.7 mm) and released close to the prepared fly antenna. A compound was categorized as biologically active if it elicited a reproducible response in the fly antenna. All flies used for the electrophysiological studies were transferred from the plastic pipette tips to vials with 76% ethanol for subsequent confirmation of species identity by morphological analysis.

Field Trapping Experiments To investigate whether *M. signaticornis* adults can be effectively attracted and collected using volatiles released by infested trees, we performed a study with available synthetic chemicals comprising 18 compounds categorized as active in GC-EAD and two additional compounds, 2-methyl-3-buten-2-ol and ipsdienol, reported to be involved in attraction of *Medetera* spp. (Hulcr et al. 2005, 2006).

For potential pest management application in the future it might be beneficial to attract *Medetera* flies to infested trees at the early stage of the beetle attack. We therefore selected synthetic mixtures of volatiles related to an early infested spruce tree for our trapping experiments. Two different quantitative compositions of the synthetic chemicals were tested: *i)* a 1:1 mix in which GC-EAD active and additional compounds were prepared in equal proportions, and *ii)* a natural mimic in which GC-EAD active and additional compounds were prepared according to the amounts released from 1 000 dm^2 of an early infested standing Norway spruce tree (Table 1). Hexane (≥ 97%, Merck, Germany) was used as diluting solvent and as control.

Trapping experiments were carried out between June and August 2019 at two different locations in Sweden affected by continuous spruce bark beetle outbreaks. These were Perstorp in Halland County (56.494°N, 13.210°E) and Asa (57.150°N, 14.765°E). At both locations, we used sticky traps that consisted of cardboard rectangles (90 cm × 30 cm) with a printed spruce bark pattern covered with transparent sticky plastic foils and fixed vertically 30–40 cm above the ground on a wooden stick (Supplementary Fig. 1). The distance between traps was around 10 m. We used two traps per synthetic mixture, with three replicate sets during the

Table 1 Purity and amounts of synthetic compounds used in the field traps

Compounds	CAS number	Purity %	Amount of compound in bait (µg/2 mL; w/v)	
			Mix 1:1	Natural mimics
2-methyl-3-buten-2-ol	115–18-4	≥97%	100	8
(±)-α-pinene	80–56-8	≥98%	100	3 432
(1*S*)-(–)-β-pinene	18,172–67-3	≥99%	100	3 462
camphene	79–92-5	≥95%	100	181
terpinolene	586–62-9	≥90%	100	129
(±)-camphor	76–22-2	≥95%	100	8
(–)-terpinen-4-ol	20126–76-5	≥95%	100	12
(1*R*)-(–)-myrtenal	57526–63-3	≥98%	100	3
(–)-*cis*-verbenol	18881–04-4	≥95%	100	12
(+)-*trans*-verbenol	473–67-6	50%	100	16
(–)-borneol	507–70-0	≥97%	100	5
(1*S*)-(–)-verbenone	1196–01-6	≥99%	100	4
(1*R*)-(–)-myrtenol	19894–97-4	≥97%	100	6
geranyl acetone	3796–70-1	≥98%	100	4
α-terpinene	99–86-5	≥94%	100	13
γ-terpinene	99–85-4	≥98.5%	100	10
(*R*)-(+)-limonene	5989–27-5	≥98%	100	252
(*S*)-(–)-limonene	5989–54-8	≥92%	100	252
α-terpineol	10482–56-1	≥98%	100	34
ipsdienol	35628–00-3	≥90%	100	2

experimental period. The traps were baited with dispensers consisting of a roll of dental cotton (0.5 cm outer diameter, 3.5 cm length; 2.75 cm^3; DAB Dental, Upplands Väsby, Sweden) that was impregnated with 2 mL of a synthetic blend and sealed inside a low-density polyethylene sachet (LDPE; 60 × 60 mm; thickness 50 µm) (Rajapack, Gothenburg, Sweden). The dispensers were fixed in the center of the sticky traps. After 24 h, the traps were checked and the numbers of *Medetera* flies and *I. typographus* beetles were counted. *Medetera* flies were separated by sex based on the hypopygium (male genital apparatus) (Supplementary Fig. 2). The *Medetera* flies were transferred to vials with 76% ethanol for subsequent identification.

Statistical Analysis All statistical analyses were performed in R studio (version 1.3.959) (Team 2020). Square roots of mean amounts of compounds were plotted in a heatmap using the function *ggplot* from the package *ggplot2*. To test for significant qualitative and quantitative differences between: *i)* volatile profiles collected from the different treatments (non-infested, infested cut, and infested standing trees); and *ii*) overall volatile profiles from infested samples over time, we used Permutational Multivariate Analysis of Variance (*PERMANOVA,* based on Bray-Curtis distances calculated from amount of compounds, 999 permutations) and pairwise *PERMANOVA* with *Bonferroni* correction for multiple testing, using the functions *adonis* and *pairwise.factorfit* from the *vegan* package in R (Oksanen et al. 2014). To visualize the differences in overall data collected, we used two different ordination methods: non-metric multidimensional scaling (*NMDS*, based on Bray–Curtis distances) from the package *vegan* with the function *metaMDS* (Bühler 2012) and principal component analysis (*PCA*) from the package *ade4* with the function *fviz_pca_ind* (Oksanen et al. 2015). To identify groups of compounds found more often in one treatment or at one site compared with another, we applied multi-level pattern analysis with the *multipatt* function from the *indicspecies* package (De Cáceres et al. 2010).

We used one-way analysis of variance (*ANOVA*) with the function *aov* to compare the number of flies collected in the traps with synthetic blends. We performed post-hoc tests with *emmeans* for pairwise multiple comparisons.

Results

Volatile Collection and Analysis A total of 118 compounds were found in 63 headspace collections from non-infested trees, infested standing trees, and infested cut trees (Supplementary Table 2). Figure 2 shows 56 of these compounds, 37 that were tentatively identified and 19 that were effectively identified. The overall volatile profiles differed significantly between non-infested and infested samples (*PERMANOVA*,

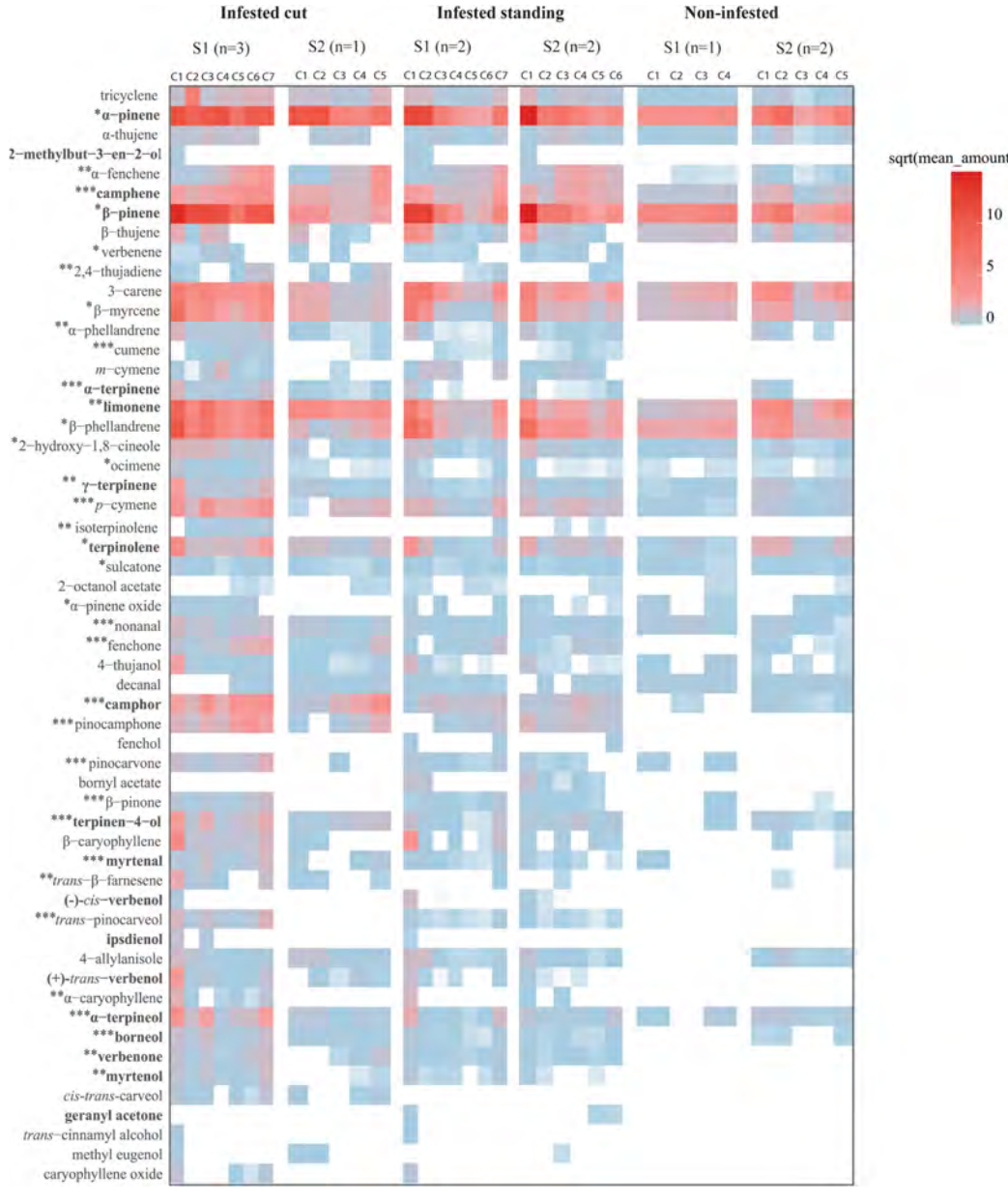

Fig. 2 Abundance (square root of the mean amount released per surface area and time ($ng(dm^2 s)^{-1}$)) of compounds detected in the headspace samples collected from cut Norway spruce trees (*Piceae abies*) infested by spruce bark beetles (*Ips typographus*), standing infested or non-infested trees. Odors were sampled from trees at two forest sites (S1 and S2) in up to seven sequential collections (C1 to C7) from 10th May (for S1) or 25th May (for S2), respectively, to 25th July 2018. The dates for the first headspace collections C1 differed slightly between sites as bark beetles were earlier active at S1 compared to S2. However, collections C1 to C6 from sites 1 and 2 correspond to similar stages of bark beetle attack. The compounds are listed in the order of their GC retention time. Asterisks (*) indicates significant differences in the abundance of compounds between the treatments (*Multipatt*, $*P \leq 0.05$; $**P < 0.01$; $***P < 0.001$); n describes the number of trees used in each site per treatment. Compounds in bold have been effectively identified with synthetic standards

P = 0.001). Within the samples from infested trees, the overall volatile profiles also differed between standing and cut trees (pairwise *PERMANOVAs,* all *Bonferroni*-corrected, P = 0.01) and changed significantly over time (*PERMANOVA*, P = 0.001). In contrast, volatile profiles of samples from non-infested trees did not differ over time (*PERMANOVA*, P > 0.1). When represented with *NMDS*, the overall headspace samples from non-infested trees were clearly separated from those from infested trees, while the samples from infested standing and infested cut trees were grouped together (Fig. 3A). This indicates that headspace samples collected from infested standing and cut trees were more similar, both qualitatively and quantitatively, than headspace samples collected from non-infested trees. Similar results were obtained in the *PCA* plot (Fig. 3B). However, principal component 1 (*PC1*), which discriminated between the treatments (42.9%), also showed that headspace samples grouped at the left-hand side of *PC1* (mainly samples of from infested cut trees) displayed much higher amounts of compounds than samples grouped at the right-hand side of *PC1* (Figs. 2 and 3B).

The overall volatile profile for non-infested trees, compared with infested trees differed in terms of amounts of released compounds (e.g., camphene, cumene, α-terpinene, p-cymene, α-terpineol, terpinen-4-ol, camphor, pinocamphone, borneol, and verbenone) (Fig. 2).

Among the headspace samples from infested trees, samples from cut trees contained significantly more hydrocarbon monoterpenes (e.g., camphene, isoterpinolene, and α-terpinene), oxygenated monoterpenes (e.g., camphor, borneol, fenchone, pinocamphone, terpinen-4-ol, pinocarvone, myrtenal, and *trans*-pinocarveol), and aldehydes (e.g., nonanal) compared with samples from standing trees (*Multipatt*, $P < 0.05$) (Fig. 2, Supplementary Table 3). Volatile compounds such as fenchol, bornyl acetate, and geranyl acetone were only found in samples collected from standing trees, while caryophyllene oxide and *cis–trans* carveol were only found in samples collected from cut trees (Fig. 2).

Pairwise comparisons of infested samples collected at different time-points showed that samples collected during late bark beetle attack phases (C5-C7) differed significantly from samples collected during earlier attack phases (C1-C2) (pairwise PERMANOVA, all Bonferroni-corrected, P = 0.01). This was confirmed by the *NMDS* and *PCA* plots (Fig. 3A, B), with samples collected at the beginning of the attack (C1-C2) mainly situated in the lower, negative part of both *NMDS*2 (Fig. 3A) and *PC2* (Fig. 3B). On the other hand, samples collected later during the attack (C5-C7) clustered more in the upper positive part of both *NMDS*2 (Fig. 3A) and *PC2* (Fig. 3B).

The variable correlation plot resulting from the *PCA* (Fig. 3C) illustrates the contribution of each compound to the variation in overall dataset (including all samples collected from non-infested and infested trees at different time-points). Compounds in the lower part of *PC2* (brown in Fig. 3C), such as α and β-caryophyllene, α-thujene, β-myrcene, and β-phellandrene, were emitted in larger amounts at the beginning of bark beetle attack, while compounds in the upper part of *PC2* (green in Fig. 3C), such as pinocarvone, myrtenal, fenchone, β-pinone, and verbenone, were emitted in larger amounts at the end of the attack (*Multipatt*, both $P < 0.05$). This indicates that emission of hydrocarbon monoterpenes was high at the beginning of bark beetle attack and that emission of oxygenated monoterpenes was high at the end of the attack (Fig. 2; Supplementary Table 3). The shift in the overall volatile composition of infested trees seemed to occur during the first 30–40 days after bark beetle attack was initiated.

We also found a significant interaction between treatment and site (*PERMANOVA*, P = 0.02). The reason was that odor samples from infested cut trees collected at Site 1 contained higher amounts of hydrocarbon and oxygenated monoterpenes than samples from infested cut trees at Site 2 (*Multipatt*, $P < 0.05$) (Fig. 2, Supplementary Table 3).

GC-EAD Responses by *Medetera signaticornis* to Early-infested Standing Spruce Odor Samples GC-EAD analysis revealed that 22 active compounds from early-infested spruce trees elicited similar antennal responses in both *M. signaticornis* males and females. The active compounds were identified and divided into three groups according to their primary source of origin (Fig. 4A, B). The first group comprised (–)-*cis*-verbenol, (+)-*trans*-verbenol, and myrtenol, which are known as compounds produced by *I. typographus* (Birgersson et al. 1984; Birgersson 1989). The second group comprised isoterpinolene, α-pinene oxide, camphor, pinocamphone, terpinen-4-ol, myrtenal, borneol, α-terpineol, verbenone and geranyl acetone, all of which except verbenene are oxygenated monoterpenes that are known to be primarily produced by microorganisms associated with *I. typographus* (Leufvén et al. 1984, 1988; Kandasamy et al. 2021). The third group comprised α-pinene, α-fenchene, β-pinene, camphene, 3-carene, limonene, γ-terpinene and terpinolene, which are hydrocarbon monoterpenes produced by the spruce host tree (Phillips and Croteau 1999; Keeling and Bohlmann 2006). We found that α-terpinene elicited antennal responses in some female *M. signaticornis*, but not consistently in all replicates. However, the antennal activity of this compound was confirmed later with a synthetic standard (data not shown).

Field Trapping Experiments Traps with either of the two synthetic blends of chemicals (mix 1:1 and natural mimic) caught significantly more *Medetera* flies than the hexane control ($F = 4.3$; $df = 2, 18$; $P < 0.05$) (Table 2). The number of flies trapped was similar for the two synthetic blends.

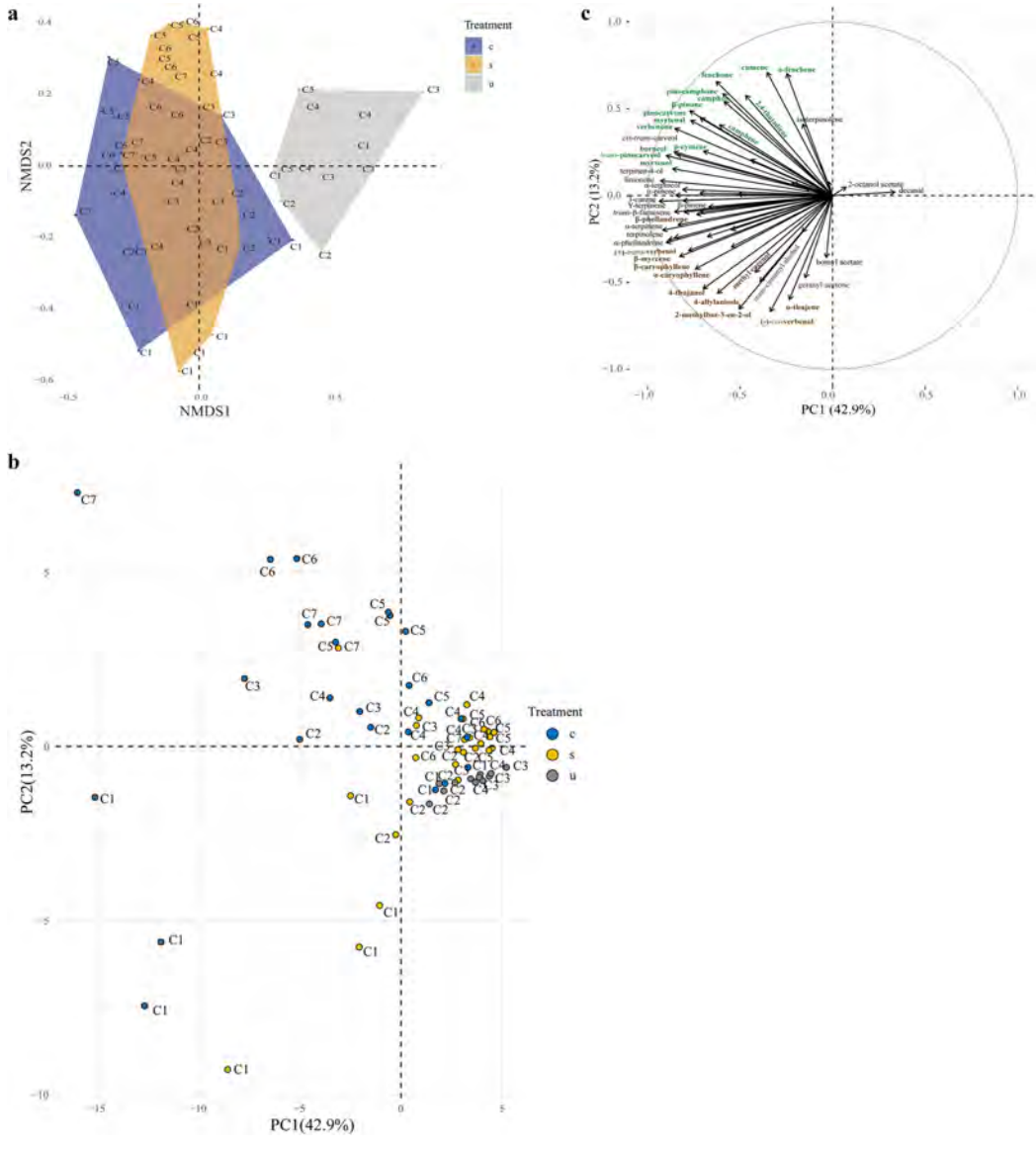

Fig. 3 Comparison between the overall volatile profiles found in the headspace samples collected from cut Norway spruce trees (*Piceae abies*) infested by bark beetles (*Ips typographus*) (c), standing infested (s) or non-infested trees (u) over time. **A**) Non-metric multidimensional scaling (*NMDS*) with two synthetic axes and a stress value lower than 0.09. **B** and **C**) Principal component analysis (*PCA*) with *PC1* and *PC2* summarizing 56.1% of the variance of the dataset. Individual samples from the differently treated trees illustrated in **A** and **B** are labeled with different colors representing bark beetle-infested cut trees (blue, $n=25$), bark beetle infested standing trees (yellow, $n=26$) and non-infested trees (grey, $n=12$). Numbers on the color-labeled points C1-7 designate the order of individual collections within the three treatments. Samples that are positioned close to each other have similar volatile profiles. The variable plot **C**) represents the contribution of individual compounds to the main variance in the dataset. Positively correlated compounds are grouped in the same quadrant; negatively correlated compounds are grouped in opposite quadrants. Compounds with long distance from the origin (long arrows) strongly contribute to the samples loaded in the same quadrant. Compounds represented in brown contribute significantly to the overall odor profiles at the beginning of the bark beetle attack (C1-C2) and compounds represented in green contribute significantly to the overall odor profiles at the end of bark beetle attack (C5-C7) (*Multipatt*, $*P \leq 0.05$; $**P < 0.01$; $***P < 0.001$)

Approximately 71% of the flies identified from traps with synthetic blends were *M. signaticornis* females, 27% were *M. signaticornis* males, and 2% were *M. ambigua* Zetterstedt (Table 2).

The number of *I. typographus* beetles was also significantly higher for both synthetic blends compared with the control ($F = 9.8$; $df = 2,18$, $P < 0.005$). The traps baited with natural mimic collected slightly more bark beetles than the traps with the 1:1 mix, but the difference was not statistically significant.

Discussion

Our aim in this study was to identify key compounds that attract the bark beetle predator *M. signaticornis* to bark beetle-infested Norway spruce trees. In field trials, we demonstrated that *M. signaticornis* females and males were attracted to synthetic blends of compounds associated with bark beetles, their symbiotic microorganisms, and host trees. Analyses of headspace samples from infested Norway spruce trees showed that headspace composition and compound concentration varied depending on the time-point of collection, apparently following different stages of bark beetle attack (early, late).

More specifically, our analyses revealed that headspace samples from early-infested trees contained high amounts of hydrocarbon monoterpenes such as α and β-caryophyllene, α-thujene, β-myrcene, and β-phellandrene, while headspace samples from late-infested trees were mainly dominated by oxygenated monoterpenes such as pinocarvone, myrtenal, fenchone, β-pinone, and verbenone. Previous studies have also found that headspace samples from bark beetle-infested logs contain a complex mixture of volatiles that changes both qualitatively and quantitatively over the different stages of bark beetle attack (Birgersson et al. 1984; Birgersson and Bergström 1989; Pettersson and Boland 2003). At early stages, we found that headspace samples from infested Norway spruce trees consisted mainly of hydrocarbon mono- and sesquiterpenes, which are released as a result of bark beetle tunneling (Phillips and Croteau 1999; Keeling and Bohlmann 2006), while in late stages of attack release of oxygenated monoterpenes increased, due to the establishment of symbiotic microorganisms such as yeasts (Birgersson et al. 1984; Leufvén et al. 1984; Leufvén and Birgersson 1987) and fungi introduced by the bark beetles (Kandasamy et al. 2016, 2021). For example, *Ophiostomatoid* fungi (*Endoconidiophora polonica*, *Grosmannia penicillata, Leptographium europhioides, Ophiostoma bicolor, O. piceae*) lining the gallery walls in bark beetle-infested trees contribute to the release of oxygenated monoterpenes such as camphor, pinocamphone, borneol, and terpinen-4-ol (Kandasamy et al. 2016, 2021). In agreement with our findings, Pettersson and Boland (2003) observed that the maximum ratio of oxygenated monoterpenes occurs in later stages of beetle attack, which coincides with the presence of late instar bark beetle larvae and appears to be an important cue for parasitoids that attack bark beetles (Pettersson 2001). In our study, we also found that infested cut trees emitted significantly higher amounts of certain volatile compounds compared to infested standing trees. According to the literature, cutting induces changes in the volatile composition, such as increased release of oxygenated monoterpenes (Strömvall and Petersson 1991; Pettersson and Boland 2003). Mechanical damage increases the release of volatile terpenes from host trees that can auto-oxidize when exposed to the air generating more oxygenated monoterpenes (Keeling and Bohlmann 2006; Benoid et al. 2021). In addition, exposed wounds can be contaminated with various types of microorganisms that can contribute to the emission of compounds from cut trees. In our study, we have not measured and compared the volatiles from non-infested cut trees and for this reason it is not possible to conclude which compounds are being produced as a direct result of the mechanical damage.

According to Hedgren et al. (2004), differences in the release rates and composition of volatiles from infested standing and infested cut trees seem to affect attraction of different *Medetera* species, with the total number of *Medetera* species emerging from bark beetle-infested standing Norway spruce trees being 10 times higher than the number emerging from infested cut trees. Moreover, some *Medetera* species were present in both standing and cut Norway spruce trees, while other species only occurred in standing or in cut infested trees. In addition to the composition and release rates of compounds from infested cut or infested standing trees, the number of prey beetles, nutritional quality of the bark and visual cues such as tree orientation, bark texture, and hardness may also influence host location and oviposition by *Medetera* flies (Lawson et al. 1996; Goyer et al. 2004).

Previous studies have shown that *M. setiventris* and *M. melancholica* are attracted to components of *I. typographus* aggregation pheromone and that the attraction increases if aggregation pheromone is combined with host tree monoterpenes (α-pinene, β-pinene, and limonene) (Rudinsky et al. 1971; Hulcr et al. 2005, 2006). However, logs from infested trees have been found to be more attractive to *M. bistriata* Parent adults than a mixture of bark beetle aggregation pheromone and tree monoterpenes, indicating that additional cues, such as volatile organic compounds produced by microbial bark beetle symbionts, might play a role in host location (Williamson 1971). *Medetera signaticornis* adults arrive at freshly attacked trees almost simultaneously with bark beetles, but have also been found on attacked trees

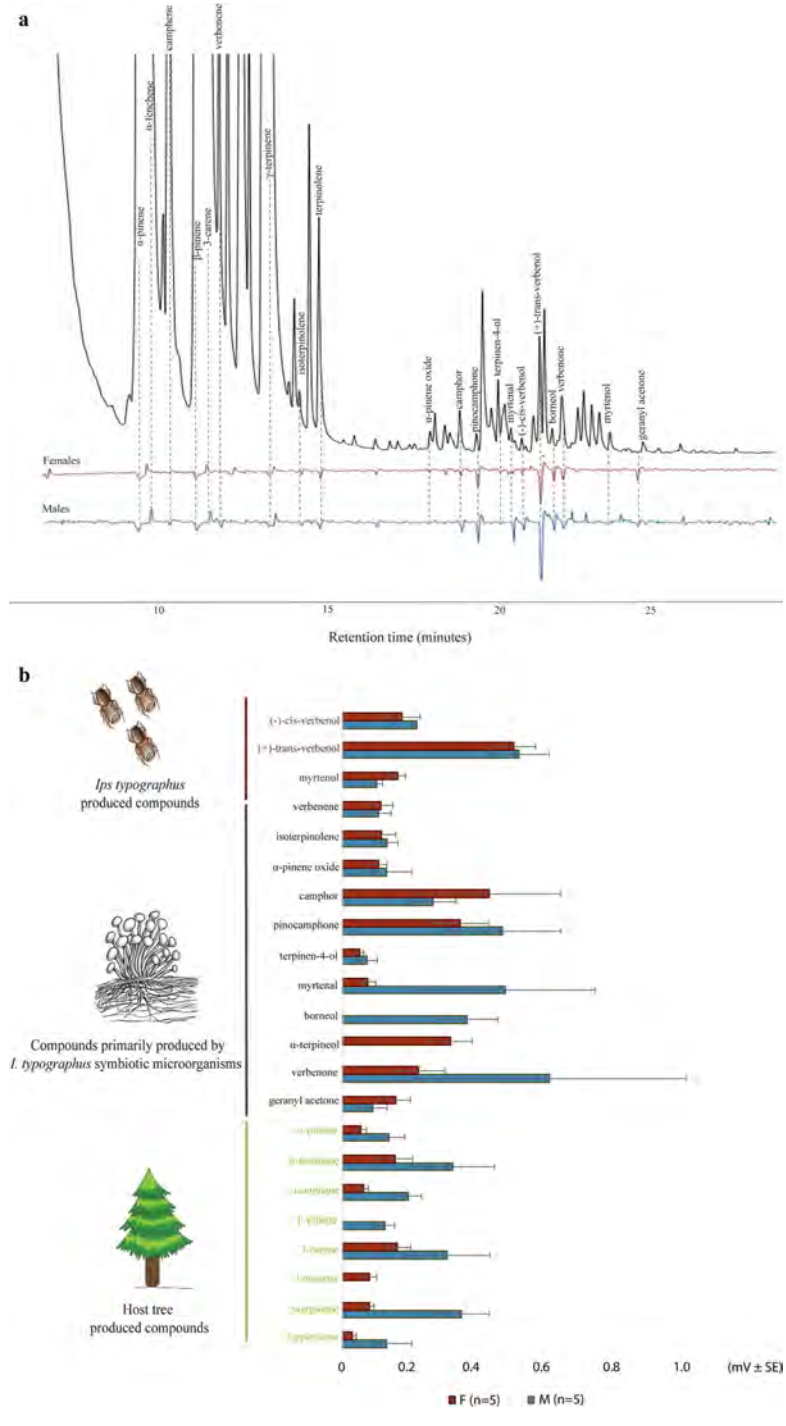

Fig. 4 GC-EAD responses of *M. signaticornis* fly antennae stimulated with odors collected from early infested Norway spruce tree. **A**) Chromatogram and electroantennograms showing the antennal stimulation of a female and a male fly antenna in response to compounds eluting from the GC. Dotted lines connect antennal responses with chromatogram peaks of active compounds. **B**) Mean response (mV ± SE) to the active compounds organized according to their source. Compounds represented in brown font are produced by the bark beetle *Ips typographus*, compounds in black font are produced by the *I. typographus* associated microorganisms and compounds in green font are produced by the Norway spruce tree (*Picea abies*). (F) females and (M) males

Table 2 Total number of *Medetera* species and *I. typographus* collected in the field traps during a 24 h trapping experiment

	Control	Mix 1:1	Natural mimics	P value
Total number of *Medetera*	54[b]	158[a]	132[ab]	F = 4.3; df = 2,18; $P < 0.05$
M. signaticornis	23F; 7 M	80F; 32 M	73F; 26 M	
M. ambigua		2F; 1 M	1F	
Unidentified	24	43	32	
Total number of Scolytidae				
I. typographus	2[b]	77[a]	106[a]	F = 9.8; df = 2,18; $P < 0.005$

Different small letters (a,b) within a row indicate significant differences between the total number of *Medetera* species or *I. typographus* individuals found in traps with the three different baits according to post-hoc tests following one-way *ANOVA*. F-values provide a measure for variation between samples, df abbreviates the degrees of freedom and $P < 0.05$ provides a measure for significant difference between means. Note that not all specimens could be identified to species level due to body damage caused from the sticky traps. (F) Females or (M) males refers to the total number of specimens collected from each sex

after emission of bark beetle pheromone has ceased (Lawson et al. 1997). Like *M. bistriata*, *M. signaticornis* adults may use other reliable host cues besides bark beetle aggregation pheromone and tree compounds.

Odors emitted from microorganisms living in symbiosis with bark beetles have been shown to impact the behavior of some unidentified *Medetera* species, which are more attracted to logs colonized by fungi (e.g., *Ophiostoma ips*) or a bacterial strain (*Burkholderia* sp.) than to uncolonized logs (Boone et al. 2008). Individuals of *M. signaticornis* are commonly found on Norway spruce trees infested with *I. typographus*, but have also been reported on other *Picea* and *Pinus* tree species infested with bark beetles from the genera *Dendroctunus*, *Dryocoetes*, *Scolytus*, and *Pityogenes* (Coleoptera: Curculionidae, Scolytinae) (Bickel 1985). Many of these bark beetle species, if not all, are associated with *Ophiostomatoid* fungi (Klepzig and Six 2004). Thus, volatiles from *Ophiostoma* fungi combined with tree-produced compounds may provide reliable cues for the predatory *M. signaticornis* to detect hosts throughout a bark beetle attack, even after pheromone production by the bark beetle has ceased. Microbial odors are important components of tritrophic interactions and may contribute to the attraction or repellence of predators and parasitoids to food sources or oviposition sites (Davis et al. 2013; Kandasamy et al. 2016).

Our GC-EAD studies on odors collected from freshly attacked spruce logs revealed that *M. signaticornis* males and females were able to detect several compounds produced by the host trees, bark beetles, and bark beetle associated microorganisms. The flies responded to (–)-*cis*-verbenol, (+)-*trans*-verbenol, and myrtenol. These three compounds are produced by the bark beetle *I. typographus* (Birgersson et al. 1984; Birgersson 1989) and are detoxification products from (±)-α-pinene (i.e. (–)-(4*S*)-*cis*-verbenol from (–)-α-pinene, (+)-(4*S*)-*trans*-verbenol from (+)-α-pinene, and myrtenol from both (+) and (–)-α-pinene), but only (–)-*cis*-verbenol is known as a pheromone component by *I. typographus* (Renwick et al. 1976; Wood 1982; Lindström et al. 1989; Blomquist et al. 2010). The flies also responded to isoterpinolene, α-pinene oxide, camphor, pinocamphone, terpinen-4-ol, myrtenal, borneol, α-terpineol, verbenone and geranyl acetone. These compounds are known to be primarily produced by microorganisms associated with *I. typographus* (Leufvén et al. 1984, 1988; Kandasamy et al. 2021). However, some compounds (e.g., terpinen-4-ol, camphor, borneol, α-terpineol and verbenone) can also be found in small amounts in the different parts (e.g., needles, bark, roots) of a healthy Norway spruce tree (Duan et al. 2020) or in other plants species (e.g., pinocamphone, camphor, borneol, α-terpineol, verbenone and geranyl acetone) (Knudsen et al. 1993). Verbenone can also be produced by many species of *Dendroctonus.* However, *Ips* beetles in general, and *I. typographus* in particular, does not produce verbenone (Francke and Vité 1983). Therefore in this case microorganisms are the most probable source of this compound. Verbenene also included in this group is not oxygenated *per se*, but may be a deoxidized product of verbenone as both compounds have a similar chemical structure and according to Blomquist et al. (2010) verbenol, verbenone and verbenene are all produced from hydroxylation of α-pinene. In addition, verbenene has not been found produced by *I. typographus* or the host tree and therefore the most probable source is microbial.

Interestingly, many of these GC-EAD active compounds are also known to be detected by other natural enemies of *I. typographus* (see Supplementary Table 4). For example, the predatory clerid beetle *Thanasimus formicarius* Linnaeus (Coleoptera: Cleridae) is attracted to bark beetle aggregation pheromone and tree monoterpenes, and possesses olfactory receptors for oxygenated monoterpenes produced by symbiotic microorganisms (e.g., camphor and pinocamphone) (Hansen 1983; Tømmerås 1985). Similarly, the Pteromalid parasitoid species *Rhopalicus tutela* Walker, *Roptrocerus mirus* Walker, and *Roptrocerus xylophagorum* Ratzeburg (Hymenoptera: Pteromalidae) respond to tree-produced compounds, but seem to be more attracted to oxygenated monoterpenes (e.g.,

camphor and pinocamphone) primarily produced by symbiotic fungi of the bark beetle (Pettersson 2001; Pettersson et al. 2001; Pettersson and Boland 2003). The detection of such microbial odors by different classes of natural enemies indicates that these may be crucial for location of bark beetles as prey. Therefore, further studies need to be performed to determine whether specific fungal compounds are necessary or sufficient to attract natural enemies such as *Medetera* flies.

Our field experiments with traps baited with synthetic blends of potential host cues revealed attraction for both sexes of *M. signaticornis*. Unsurprisingly, females, which use spruce trees for oviposition, were attracted in higher numbers than males. It is unclear why males are attracted to bark beetle-infested trees, but they are possibly used as meeting and mating sites by both sexes of *M. signaticornis* (Hopping 1947). Individuals of *M. ambigua* were also found in the traps, indicating that attraction was not limited to *M. signaticornis*. In the future, we will examine in more detail the attractiveness of synthetic blends for *M. signaticornis* and other *Medetera* species.

In this study, we tested and confirmed the hypothesis that *M. signaticornis* adult flies use multiple semiochemicals to detect bark beetle-infested Norway spruce trees throughout infestation. Male and female flies responded both electrophysiologically and behaviorally to several compounds emitted from host trees, bark beetles, and symbiotic microorganisms. Besides tree-produced compounds, oxygenated monoterpenes produced by symbiotic microorganisms may be a reliable cue for the predatory *M. signaticornis*, especially in the later stages of bark beetle attack when production of bark beetle pheromone has declined. Thus, the multitrophic interaction between predatory *M. signaticornis*, bark beetle, host tree, and microorganisms needs to be assessed in future studies on management and ecology of bark beetles and their natural enemies. The present study provides a sound foundation for further field work aiming to adjust the attractiveness of the synthetic blend to mimic relevant host cues. Such a blend could be used to monitor *Medetera* flies or to attract more flies to newly infested areas, increasing biological control and reducing the number of bark beetles emerging from infested trees, which in turn would reduce the economic losses to the forest sector.

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Data Availability All of the data on which conclusions rely in this study are included in this published article and its supplementary information files.

Declarations

Competing interests The authors declare no competing interests.

References

Anon (2023) Reference climate data from the Unit for Field-based Forest Research climate monitoring program. University of Agricultural Sc. [Downloaded from https://www.slu.se/esf-referenceclimate/, 2023-01-10]

Beaver RA (1966) The biology and immature stages of two species of *Medetera* (Diptera: Dolichopodidae) associated with the bark beetle *Scolytus scolytus* (F.). Proceedings of the Royal Entomological Society of London. Series A, General Entomology. Wiley Online Library, pp 145–154. https://doi.org/10.1111/j.1365-3032.1966.tb00334.x

Benoid R, Belhadj N, Lailliau M, Dagaut P (2021) Autoxidation of terpenes a common pathway in trophospheric and low temperature combustion conditions: the case of limonene and α-pinene. Atmos Chem Phys Discuss [preprint]. https://doi.org/10.5194/acp-2021-964

Bentz BJ, Jönsson AM, Schroeder M, Weed A, Wilcke RAI, Larsson K (2019) *Ips typographus* and *Dendroctonus ponderosae* models project thermal suitability for intra-and inter-continental establishment in a changing climate. Front Forests Global Change 2:1. https://doi.org/10.3389/ffgc.2019.00001

Bickel DJ (1985) A revision of the nearctic *Medetera* (Diptera: Dolichopodidae), United States Department of Agriculture, Agricultural Research Service. Technical Bulletin Number 1692

Birgersson G (1989) Host tree resistance influencing pheromone production in *Ips typographus* (Coleoptera: Scolytidae). Ecography 12:451–456. https://doi.org/10.1111/j.1600-0587.1989.tb00922.x

Birgersson G, Bergström G (1989) Volatiles released from individual spruce bark beetle entrance holes quantitative variations during the first week of attack. J Chem Ecol 15:2465–2483. https://doi.org/10.1007/BF01020377

Birgersson G, Schlyter F, Löfqvist J, Bergström G (1984) Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases. J Chem Ecol 10:1029–1055. https://doi.org/10.1007/BF00987511

Blomquist GJ, Figueroa-Teran R, Aw M, Song M, Gorzalski A, Abbott NL, Chang E, Tittiger C (2010) Pheromone production in bark beetles. Insect Biochem Mol Biol 40:699–712. https://doi.org/10.1016/j.ibmb.2010.07.013

Boone CK, Six DL, Zheng Y, Raffa KF (2008) Parasitoids and dipteran predators exploit volatiles from microbial symbionts to locate bark beetles. Environ Entomol 37:150–161. https://doi.org/10.1093/ee/37.1.150

Bühler A (2012) NMDS Tutorial in R. https://jonlefcheck.net/2012/10/24/nmds-tutorial-in-r/. Accessed 22 Aug 2022

Davis TS, Crippen TL, Hofstetter RW, Tomberlin JK (2013) Microbial volatile emissions as insect semiochemicals. J Chem Ecol 39:840–859. https://doi.org/10.1007/s10886-013-0306-z

De Cáceres M, Legendre P, Moretti M (2010) Improving indicator species analysis by combining groups of sites. Oikos 119:1674–1684. https://doi.org/10.1111/j.1600-0706.2010.18334.x

Duan Q, Bonn B, Kreuzwieser J (2020) Terpenoids are transported in the xylem sap of Norway spruce. Plant Cell Environ 43:1766–1778. https://doi.org/10.1111/pce.13763

Edmonds RL, Eglitis A (1989) The role of the Douglas-fir beetle and wood borers in the decomposition of and nutrient release from Douglas-fir logs. Can J for Res 19:853–859. https://doi.org/10.1139/x89-130

El-Sayed A (2016) The pherobase: database of pheromones and semiochemicals. https://www.pherobase.com/database/compound/compounds-index.php. Accessed Apr 2022

Fitzgerald T, Nagel W (1972) Oviposition and larval bark-surface orientation of *Medetera aldrichii* (Diptera: Dolichopodidae): response to a prey-liberated plant terpene. Ann Entomol Soc Am 65:328–330. https://doi.org/10.1093/aesa/65.2.328

Francke W, Vité JP (1983) Oxygenated monoterpenes in pheromone systems of bark beetles 1. Zeitschrift Für Angew Entomol 96:146–156. https://doi.org/10.1111/j.1439-0418.1983.tb03655.x

Ghimire RP, Kivimäenpää M, Blomqvist M, Holopainen T, Lyytikäinen-Saarenmaa P, Holopainen JK (2016) Effect of bark beetle (*Ips typographus* L.) attack on bark VOC emissions of Norway spruce (*Picea abies* Karst.) trees. Atmos Environ 126:145–152. https://doi.org/10.1016/j.atmosenv.2015.11.049

Gomez-Gallego M, Galiano L, Martínez-Vilalta J, Stenlid J, Capador-Barreto HD, Elfstrand M, Camarero JJ, Olivia J (2022) Interaction of drought- and pathogen-induced mortality in Norway spruce and Scots pine. Plant Cell Environ 45:2292–2305. https://doi.org/10.1111/pce.14360

Goyer R, Lenhard G, Strom BL (2004) The influence of silhouette color and orientation on arrival and emergence of *Ips* pine engravers and their predators in loblolly pine. For Ecol Manag 191:147–155. https://doi.org/10.1016/j.foreco.2003.11.012

Grégoire J-C, Raffa KF, Lindgren BS (2015) Bark Beetles: Biology and Ecology of Native and Invasive Species. Chapter 1, Elsevier, pp 1–40. https://doi.org/10.1016/B978-0-12-417156-5.00001-0

Gunulf A, Wang L, Englund J-E, Rönnberg J (2013) Secondary spread of *Heterobasidion parviporum* from small Norway spruce stumps to adjacent trees. For Ecol Manag 287:1–8. https://doi.org/10.1016/j.foreco.2012.09.011

Hammerbacher A, Schmidt A, Wadke N, Wright LP, Schneider B, Bohlmann J, Brand WA, Fenning TM, Gershenzon J, Paetz C (2013) A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce. Plant Physiol 162:1324–1333. https://doi.org/10.1104/pp.113.218610

Hannerz M, Thorsén Å, Mattsson S, Weslien J (2002) Pine weevil (*Hylobius abietis*) damage to cuttings and seedlings of Norway spruce. For Ecol Manag 160:11–17. https://doi.org/10.1016/S0378-1127(01)00467-4

Hansen K (1983) Reception of bark beetle pheromone in the predaceous clerid beetle, *Thanasimus formicarius* (Coleoptera: Cleridae). J Comp Physiol 150:371–378. https://doi.org/10.1007/BF00605026

Hedgren PO, Schroeder LM (2004) Reproductive success of the spruce bark beetle Ips typographus (L.) and occurrence of associated species: a comparison between standing beetle-killed trees and cut trees. For Ecol Manag 203:241–250. https://doi.org/10.1016/j.foreco.2004.07.055

Hlásny T, Zimova S, Merganicova K, Stepanek P, Modlinger R, Turcani M (2021) Devastating outbreak of bark beetles in the Czech Republic: drivers, impacts, and management implications. For Ecol Manag 490:119075. https://doi.org/10.1016/j.foreco.2021.119075

Holopainen JK, Virjamo V, Ghimire RP, Blande JD, Julkunen-Tiitto R, Kivimäenpää M (2018) Climate change effects on secondary compounds of forest trees in the northern hemisphere. Front Plant Sci 9:1–10. https://doi.org/10.3389/fpls.2018.01445

Hopping GR (1947) Notes on the seasonal development of *Medetera aldrichii* Wheeler (Diptera: Dolichopodidae) as a predator of the douglas fir bark-beetle, *Dendroctonus Pseudotsugae* Hopkins L. Can Entomol 79:150–153. https://doi.org/10.4039/Ent79150-7

Hulcr J, Pollet M, Ubik K, Vrkoc J (2005) Exploitation of kairomones and synomones by *Medetera* spp.(Diptera: Dolichopodidae), predators of spruce bark beetles. Eur J Entomol 102:655–662

Hulcr J, Ubik K, Vrkoc J (2006) The role of semiochemicals in tritrophic interactions between the spruce bark beetle *Ips typographus*, its predators and infested spruce. J Appl Entomol 130:275–283. https://doi.org/10.1111/j.1439-0418.2006.01069.x

Juráň S, Pallozzi E, Guidolotti G, Fares S, Šigut L, Calfapietra C, Alivernini A, Savi F, Večeřová K, Křůmal K, Večeřa Z, Urban O (2017) Fluxes of biogenic volatile organic compounds above temperate Norway spruce forest of the Czech Republic. Agr for Meteorol 232:500–513. https://doi.org/10.1016/j.agrformet.2016.10.005

Kandasamy D, Gershenzon J, Hammerbacher A (2016) Volatile organic compounds emitted by fungal associates of conifer bark beetles and their potential in bark beetle control. J Chem Ecol 42:952–969. https://doi.org/10.1007/s10886-016-0768-x

Kandasamy D, Zaman R, Nakamura Y, Zhao T, Hartmann H, Andresson MN, Hammerbacher A, Gershenzon J (2021) Bark beetles locate fungal symbionts by detecting volatile fungal metabolites of host tree resin monoterpenes. BioRxiv. https://doi.org/10.1101/2021.07.03.450988

Kärvemo S, Schroeder LM (2010) A comparison of outbreak dynamics of the spruce bark beetle in Sweden and the mountain pine beetle in Canada (Curculionidae: Scolytinae). Ent Tidskr 131:215–224

Keeling CI, Bohlmann J (2006) Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. New Phytol 170:657–675. https://doi.org/10.1111/j.1469-8137.2006.01716.x

Kenis M, Wermelinger B, Grégoire J-C (2007) Research on Parasitoids and Predators of Scolytidae – A Review. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans HF (eds) Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-2241-8_11

Klepzig KD, Six D (2004) Bark beetle-fungal symbiosis: context dependency in complex associations. Symbiosis 37:189–205

Knudsen JT, Tollsten L, Bergström G (1993) Floral scents – a checklist of volatile compounds isolated by headspace techniques. Phytochemistry 33:253–280

Lawson SA, Furuta K, Katagiri K (1996) The effect of host tree on the natural enemy complex of *Ips typographus japonicus* Niijima (Col., Scolytidae) in Hokkaido, Japan. J Appl Entomol 120:77–86. https://doi.org/10.1111/j.1439-0418.1996.tb01570.x

Lawson SA, Furuta K, Katagiri K (1997) Effect of natural enemy exclusion on mortality of *Ips typographus japonicus* Niijima (Col., Scolytidae) in Hokkaido, Japan. J Appl Entomol 121:89–98. https://doi.org/10.1111/j.1439-0418.1997.tb01376.x

Leufvén A, Birgersson G (1987) Quantitative variation of different monoterpenes around galleries of *Ips typographus* (Colleoptera:

Scolytidae) attacking Norway spruce. Canad J Bot 65:1038–1044. https://doi.org/10.1139/b87-144

Leufvén A, Bergström G, Falsen E (1984) Interconversion of verbenols and verbenone by identified yeasts isolated from the spruce bark beetle *Ips typographus*. J Chem Ecol 10:1349–1361. https://doi.org/10.1007/BF00988116

Leufvén A, Bergström G, Falsen E (1988) Oxygenated monoterpenes produced by yeasts, isolated from *Ips typographus* (Coleoptera: Scolytidae) and grown in phloem medium. J Chem Ecol 14:353–362. https://doi.org/10.1007/BF01022551

Lindström M, Torbjörn N, Göran B, Fredrik S (1989) Variation of enantiomeric composition of α-pinene in norway spruce, *Picea abies*, and its influence on production of verbenol isomers by Ips typographus in the field. J Chem Ecol 15:541–548. https://doi.org/10.1007/BF01014699

Marini L, Økland B, Jönsson AM, Bentz B, Carroll A, Forster B, Grégoire JC, Hurling R, Nageleisen LM, Netherer S (2017) Climate drivers of bark beetle outbreak dynamics in Norway spruce forests. Ecography 40:1426–1435. https://doi.org/10.1111/ecog.02769

Martin DM, Gerhenzon J, Bohlmann J (2003) Induction of volatile terpene biosynthesis and diurnal emission by methyl jasmonate in foliage of Norway spruce. Plant physiol 132:1586–1599 (http://www.plantphysiol.org/cgi/doi/10.1104/pp.103.021196)

Nicolai V (1995) The impact of *Medetera dendrobaena* Kowarz (Dipt., Dolichopodidae) on bark beetles. J Appl Entomol 119:161–166. https://doi.org/10.1111/j.1439-0418.1995.tb01264.x

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P, O'Hara R, Simpson G, Solymos P, Stevens M, Wagner H (2014) Vegan: Community ecology package. R package version 2.2-.0. http://CRAN.Rproject.org/package=vegan. Accessed May 2022

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P, O'Hara R, Simpson G, Solymos P, Henry M, Stevens M (2015) Vegan community ecology package: ordination methods, diversity analysis and other functions for community and vegetation ecologists. R package 2.6–2. http://CRAN.Rproject.org/package=vegan. Accessed May 2022

Ounap H (2001) Insect predators and parasitoids of bark beetles (Col., Scolytidae) in Estonia. PhD Thesis, Institute of Plant Protection, Faculty of Agronomy, Estonian Agricultural University, Estonia

Pettersson EM (2001) Volatile attractants for three Pteromalid parasitoids attacking concealed spruce bark beetles. Chemoecology 11:89–95. https://doi.org/10.1007/PL00001837

Pettersson EM, Boland W (2003) Potential parasitoid attractants, volatile composition throughout a bark beetle attack. Chemoecology 13:27–37. https://doi.org/10.1007/s000490300003

Pettersson EM, Birgersson G, Witzgall P (2001) Synthetic attractants for the bark beetle parasitoid *Coeloides bostrichorum* Giraud (Hymenoptera: Braconidae). Naturwissenschaften 88:88–91. https://doi.org/10.1007/s001140100209

Pettersson EM (2000) Vital volatiles-host location in parasitic wasps attacking bark beetles. PhD Thesis, Institute of Chemical Ecology, University of Gothenburg, Sweden

Phillips MA, Croteau RB (1999) Resin-based defenses in conifers. Trends Plant Sci 4:184–190. https://doi.org/10.1016/s1360-1385(99)01401-6

Renwick J, Hughes P, Krull I (1976) Selective production of cis-and transverbenol from (-)-and (+)-α-pinene by a bark beetle. Science 191:199–201. https://doi.org/10.1126/science.1246609

Romashkin I, Neuvonen S, Tikkanen O-P (2020) Northward shift in temperature sum isoclines may favour *Ips typographus* outbreaks in European Russia. Agric For Entomol 22:238–249. https://doi.org/10.1111/afe.12377

Rouault G, Candau J-N, Lieutier F, Nageleisen L-M, Martin J-C, Warzée N (2006) Effects of drought and heat on forest insect populations in relation to the 2003 drought in Western Europe. Ann for Sci 63:613–624. https://doi.org/10.1051/forest:2006044

Rudinsky J, Novak V, Švihra P (1971) Attraction of the Bark Beetle *Ips typographus* L. to terpenes and a male-produced pheromone. J Appl Entomol 67:179–188

Schlyter F, Birgersson G, Byers JA, Löfqvist J, Bergström G (1987) Field response of spruce bark beetle, *Ips typographus,* to aggregation pheromone candidates. J Chem Ecol 13:701–716. https://doi.org/10.1007/BF01020153

Seybold SJ, Huber DPW, Lee JC, Graves AD, Bohlmann J (2006) Pine monoterpenes and pine bark beetle: a marriage of convenience for defense and chemical communication. Phytochem Rev 5:143–178. https://doi.org/10.1007/s11101-006-9002-98

Stadelmann G, Bugmann H, Wermelinger B, Bigler C (2014) Spatial interactions between storm damage and subsequent infestations by the European spruce bark beetle. For Ecol Manag 318:167–174. https://doi.org/10.1016/j.foreco.2014.01.22

Strömvall AM, Petersson G (1991) Conifer monoterpenes emitted to air by logging operations. Scand J for Res 6:253–258. https://doi.org/10.1080/02827589109382666

Team R (2020) RStudio: Integrated Development for R. RStudio, PBC, Boston URL http://www.rstudio.com/. Accessed Apr 2022

Tømmerås BÅ (1985) Specialization of the olfactory receptor cells in the bark beetle *Ips typographus* and its predator *Thanasimus formicarius* to bark beetle pheromones and host tree volatiles. J Comp Physiol 157:335–341. https://doi.org/10.1007/BF00618123

Ulrich H (2004) Predation by adult Dolichopodidae (Diptera): a review of literature with an annotated prey-predator list. Studia Dipterologica 11(2):369–403

Wadke N, Kandasamy D, Vogel H, Lah L, Wingfield BD, Paetz C, Wright LP, Gershenzon J, Hammerbacher A (2016) The bark-beetle-associated fungus *Endoconidiophora polonica*, utilizes the phenolic defense compounds of its host as a carbon source. Plant Physiol 171:914–931. https://doi.org/10.1104/pp.15.01916

Wegensteiner R, Wermelinger B, Herrmann M (2015) Bark Beetles: Biology and Ecology of Native and Invasive Species. Chapter 7, Elsevier, pp 247–304. https://doi.org/10.1016/B978-0-12-417156-5.00007-1

Wermelinger B (2002) Development and distribution of predators and parasitoids during two consecutive years of an *Ips typographus* (Col., Scolytidae) infestation. J Appl Entomol 126:521–527. https://doi.org/10.1046/j.1439-0418.2002.00707.x

Wermelinger B (2004) Ecology and management of the spruce bark beetle *Ips typographus*—a review of recent research. For Ecol Manag 202:67–82. https://doi.org/10.1016/j.foreco.2004.07.018

Williamson D (1971) Olfactory Discernment of Prey by *Medetera bistriata* (Diptera: Dolichopodidae). Ann Entomol Soc Am 64:586–589

Wood DL (1982) The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Ann Rev Entomol 27:411–446. https://doi.org/10.1146/annurev.en.27.010182.002211

Zhao T, Kandasamy D, Krokene P, Chen J, Gershenzon J, Hammerbacher A (2019) Fungal associates of the tree-killing bark beetle, *Ips typographus* vary in virulence, ability to degrade and influence bark beetle tunneling behavior. Fungal Ecol 38:71–79. https://doi.org/10.1016/j.funeco.2018.06.003

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Tree-killing bark beetles are causing high economic and ecological damage in forests worldwide. Several long-legged fly species of *Medetera* genus are important natural enemies of tree-killing bark beetles, and thus of interest as biological control agents. This thesis investigates the morphology of olfactory organs and chemical attractants used by *Medetera* flies to detect Norway spruce trees infested by the Eurasian bark beetle *I. typographus*. Prey associations of *Medetera* were reviewed and DNA barcoding was assessed for congruence with morphological identifications.

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