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Behavioral responses of predatory flies of the genus *Medetera* Fischer von Waldheim (Diptera: Dolichopodidae) and the tree-killing beetle *Ips typographus* L. (Coleoptera: Scolytinae) to odor compound blends

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Abstract

Key Message *Medetera* (Fischer von Waldheim) flies, natural enemies of the spruce bark beetle *Ips typographus* (L.), were attracted to synthetic blends of compounds produced by infested spruce trees. A subset of trapped specimens revealed sixteen *Medetera* species. Most abundant were *M. signaticornis*, *M. infumata*, and *M. prjachinae*. Only blends containing beetle-produced compounds significantly attracted *Medetera* spp. and *I. typographus*.

Context Fly species of the genus *Medetera* (Fischer von Waldheim) (Diptera: Dolichopodidae) represent one of the most important groups of natural enemies of the Eurasian bark beetle *Ips typographus* (L.), which infests Norway spruce *Picea abies* (L.) Karst. In a previous study, we showed that adult *Medetera* flies exploit semiochemicals to find beetle-infested trees however, the exact nature of those attractive compounds has not yet been determined.

Aims The aim of this follow-up study was to investigate the behavioral responses of *Medetera* spp. and *I. typographus*, to different combinations of semiochemicals.

Methods In this study, 22 volatile compounds identified from *I. typographus*-infested Norway spruce were divided into five groups (A–E) based on being primarily produced by the bark beetle *I. typographus* (group A), bark beetle-associated microorganisms (groups B and C), or spruce tree (groups D and E). The effect of the compounds in these groups in the attraction of *Medetera* species and *I. typographus* was tested in two different subtractive field trapping assays.

Results In the first subtractive assay, the full blend (ABCDE), and the blends lacking microbial compounds of group C, or spruce tree compounds of group D led to significant attraction of *Medetera* flies. Morphological identification of a subset of the specimens collected revealed that sixteen species were attracted to the synthetic blends, with *M. signaticornis* Loew being the most abundant. In the second subtractive assay, high attraction of *Medetera* flies and *I. typographus* was found for a 12-component synthetic blend.

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Conclusion The insights gained provide a basis for developing synthetic attractants to facilitate monitoring of *Medetera* flies. Future testing and optimization of these attractants will enhance our ability to monitor, conserve and utilize *Medetera* flies, thereby enabling us to better protect forests from the damaging effects of spruce bark beetles.

Keywords Biological control, Conservation biocontrol, Chemical ecology, Sustainable forestry, Bark beetle natural enemies, Long-legged flies

1 Introduction

The Eurasian spruce bark beetle *Ips typographus* (L.) (Coleoptera: Curculionidae: Scolytinae) is a major insect pest of mature Norway spruce (*Picea abies* (L.) Karst. (Pinales: Pinaceae)) and kills high numbers of trees during epidemic outbreaks. These outbreaks are usually triggered by large-scale disturbances of the forest ecosystem, including severe storms, above-average temperatures, or prolonged drought episodes (Kausrud et al. 2012; Marini et al. 2017; Hlásny et al. 2019). At present, pest management of *I. typographus* includes strategies to detect and reduce epidemic outbreaks by decreasing beetle population densities and preventing attacks on living trees (Marini et al. 2017; Wermelinger 2004). Trap-based monitoring programs use synthetic versions of bark beetle aggregation pheromones (Hansen et al. 2006; Gitau et al. 2013; Heber et al. 2021). Other pest management measures focus on harvesting windthrown timber to remove breeding substrates and debarking or cutting of infested standing trees (Stadelmann et al. 2013). However, removing infested trees may cause loss of breeding sites for beneficial insects and other animals, reduce the availability of food sources, alter the microclimate, and thus negatively affect natural enemies of *I. typographus* and species biodiversity in general (Martikainen et al. 1999; Aukema et al. 2000; Thorn et al. 2016, 2018; Leverkus et al. 2020; Vogel et al. 2021; Cours et al. 2023). In addition, these pest management measures only work when carried out thoroughly and early in the season, before the emergence of adult beetles. It has been suggested that the optimal period for salvage harvesting of windthrown logs is between the time of infestation and emergence of the first generation of the bark beetles (Wichmann and Ravn 2001; Wermelinger 2004).

Over recent decades, the benefit of natural enemies for management of *I. typographus* has been recognized, but practical implementation of biological controls is still rare (Kenis et al. 2007; Trigos-Peral et al. 2021). Natural enemies of bark beetles such as parasitoids and predators can significantly reduce the propagation of spruce bark beetle populations (Weslien and Regnander 1992; Schroeder and Weslien 1994; Schroeder 1996). Among the most important natural enemies of bark beetles are predatory long-legged fly species within the genus *Medetera* Fisher von Waldheim (Diptera: Dolichopodidae) (Lawson et al. 1996; Wermelinger 2002; Hedgren and Schroeder 2004). The adult fly females are attracted to bark beetle infested trees and oviposit near the entrance of bark beetle galleries. Upon hatching, the larvae move into the galleries to prey on the bark beetle brood including eggs, larvae, pupae, and newly emerged callow beetles that are still concealed in the galleries and pupal chambers (Beaver 1966; Nagel & Fitzgerald 1975; Bickel 1985). The efficacy of *Medetera* larvae is noteworthy, as a single larva can consume between five to 20 bark beetle individuals during development. *Medetera* spp. can reach emergence abundances of approximately 100 specimens per m^2 of spruce bark infested with bark beetles (Beaver 1966; Nicolai 1995). *Medetera* flies together with the parasitoid wasps of the genus *Roptrocerus* can contribute to more than 80% of bark beetle mortality (Wermelinger 2002).

The difficulty of identifying *Medetera* species makes studies on their biodiversity or ecological importance challenging. Identification of *Medetera* flies based on morphological characters generally requires the involvement of experienced specialized taxonomists and possibly reference insect collections with reliably identified specimens of different species. In practice, *Medetera* fly specimens are primarily identified based on the fine structure of male genital morphology, while there are almost no available morphological keys for females (Pollet et al. 2011, 2022). DNA barcoding could facilitate identification, monitoring and cataloging of *Medetera* spp. in the future. Moreover, the development of attractive baits for trapping and monitoring *Medetera* flies could help to inventory the presence, abundance, and diversity of species. More specifically, monitoring of *Medetera* could support decision making for protecting beneficial species and taking measures of sustainable forest management and conservation biological control targeting *I. typographus*. A goal of this study therefore was to create a synthetic chemical attractant that could facilitate *Medetera* trapping and monitoring.

In a previous study, using classical experimental chemical ecology, including chemo-analytical, electrophysiological, and behavioral studies of headspace collections from *I. typographus*-infested Norway spruce trees, we have demonstrated that adult flies of *Medetera signaticornis* Loew 1857 were significantly attracted to a complex synthetic blend made of 18 antennal active compounds and two additional compounds: 2-methyl-3-buten-2-ol and ipsdienol (Sousa et al. 2023a). In the current study, we hypothesized that only a fraction of these compounds significantly influences the attraction of *M. signaticornis* and possibly other *Medetera* spp. to bark beetle infested trees. To test this hypothesis, we conducted two subtractive trapping assays in spruce forest with bark beetle infested trees. First, groups of synthetic compounds were differently combined and tested for trapping *Medetera* and *I. typographus*. Second, certain components were, based on the trapping results of the first assay, removed from the blend and remaining compounds were arranged in different test combinations.

2 Material and methods

2.1 Chemicals

Twenty compounds that, when combined to a mixture, were previously found to attract *M. signaticornis* flies (Sousa et al. 2023a), and two additional isomers of the components ((+)-terpinen-4-ol and (+)-borneol) were used in this study (Table 1). Of these 22 compounds, five groups (A–E) of four to five components were created for further combinatorial testing. For generating these groups, we followed a similar strategy as in our previous study (Sousa et al. 2023a). The compounds were first attributed to three categories according to their primary biological origin (*I. typographus*, *I. typographus*-associated microorganisms, spruce tree). The compounds were further grouped in order of their Kováts retention indices if more than five components were part of the same category. Compounds produced by the bark beetle were assigned to group A and comprised the two components of the *I. typographus* aggregation pheromone: 2-methyl-3-buten-2-ol (abbreviation MB) and (–)-*cis*-verbenol (cV), and also (+)-*trans*-verbenol (tV), (–)-myrtenol (Mt), and (±)-ipsdienol (Id), which are known being produced by bark beetles during infestation (Birgersson et al. 1984; Birgersson 1989) (Table 1). Compounds primarily produced by bark beetle symbiotic microorganisms were assigned to groups B and C (Table 1). Those in group B comprised (±)-camphor (Camp), (–)-terpinen-4-ol ((–)-T4ol), (+)-terpinen-4-ol ((+)-T4ol), (–)-borneol ((–)-Bor), and (+)-borneol ((+)-Bor), while those assigned to group C were (–)-myrtenal (Mtal), (–)-verbenone (Vn), α-terpineol (αT), and geranyl acetone (GA) (Leufvén et al. 1984, 1988; Kandasamy et al. 2016; Kandasamy et al. 2023) (Table 1). Compounds produced by spruce trees were assigned to groups D and E. Accordingly, (±)-α-pinene (αP), (–)-β-pinene (βP), camphene (Cam), and terpinolene (Terp) were assigned to group D, while α-terpinene (αTerp), γ-terpinene (γT) (–)-limonene ((–)-Lim), and (+)-limonene ((+)-Lim) were assigned to group E (Keeling and Bohlmann 2006; Phillips and Croteau 1999) (Table 1). Important to note, the attribution of compounds to their primary biological origin was not strictly categorical. For example, some microbial compounds assigned to groups B and C can also be produced by Norway spruce trees, however in very small amounts (Duan et al. 2020), and the aggregation componente MB, de novo produced by the bark beetle *I. typographus*, can also be produced by the bark beetle associated microorganisms (Zhao et al. 2015; Kandasamy et al. 2019). Generally, organisms of all kingdoms release and share volatile organic compounds in common and categorization often reflects simplification and pragmatic purpose (Becher et al. 2018; Beran et al. 2019; Vlot and Rosenkranz 2022).

The amounts of compounds used in the subtractive bioassays corresponded to the amounts released from a 14-m^2 area (equivalent to a ~ 15-m high tree trunk with a ~ 30-cm breast height diameter, BHD) of a living infested Norway spruce tree during the initial stages of an *I. typographus* attack, similar as quantified in our previous study (Sousa et al. 2023a). Briefly, healthy standing trees across different forest sites had been baited with synthetic *I. typographus* aggregation pheromone (Pheroprax®, BASF, Limburgerhof, Germany) to induce controlled *I. typographus* attacks (Sousa et al. 2023a). Upon the initiation of gallery excavation by *I. typographus* beetles in the baited trees, the synthetic baits were removed. Subsequently, we collected volatiles emanating from a specified bark surface area using an adsorbent Porapak Q column. The collected samples had then been analyzed through Gas Chromatography-Mass Spectrometry (GC–MS), utilizing a fused silica column coated with DB-Wax (polyethylene glycol, df = 0.25 µm, Agilent Technologies). Compound identification had been accomplished by comparing the obtained mass spectra and Kováts retention indices against reference libraries. Quantification of compounds in the samples had been carried out using heptyl acetate (100 ng µL − 1) as an internal standard (for more details see Sousa et al. 2023a).

Table 1 Biological attribute, grouping, purity and amounts (µg) of synthetic compounds used in field traps. Synthetic blends with compounds (designed ABCDE, BCDE, ACDE, ABDE, ABCE, ABCD) were formulated in 2 mL heptane and released during 48-h experiments. Heptane was used as a control. The released rates were calculated based on the total evaporation and concentration of compounds in solution over 48-h. Abbr = abbreviation

Biological attribute (origin)	Group	Compounds	Abbr	Cas number	Purity %	Synthetic blends (µg/2 mL; w/v)							Released rate, pg/s
						Full (ABCDE)	BCDE	ACDE	ABDE	ABCE	ABCD	Control	
Compounds produced by the bark beetle *I. typographus*	A	2-methyl-3-buten-2-ol	MB	115–18–4	≥ 97%	2890	–	2890	2890	2890	2890	–	70
		(–)-*cis*-verbenol	cV	18881–04–4	≥ 95%	480	–	480	480	480	480	–	115
		(+)-*trans*-verbenol	tV	473–67–6	50%	6 393	–	6 393	6 393	6 393	6 393	–	155
		(–)-myrtenol	Mt	19894–97–4	≥ 99%	2 473	–	2 473	2 473	2 473	2 473	–	60
		(±)-ipsdienol	Id	35628–00–3	≥ 90%	782	–	782	782	782	782	–	20
Compounds primarily produced by bark beetle associated microorganisms	B	(±)-camphor	Camp	76–22–2	≥ 95%	3 333	3 333	–	3 333	3 333	3 333	–	80
		(–)-terpinen-4-ol	(–)-T4ol	20126–76–5	≥ 95%	2 518	2 518	–	2 518	2 518	2 518	–	60
		(+)-terpinen-4-ol	(+)-T4ol	2438–10–0	≥ 98,5%	2 518	2 518	–	2 518	2 518	2 518		60
		(–)-borneol	(–)-Bor	507–70–0	≥ 97%	1 017	1 017	–	1 017	1 017	1 017	–	25
		(+)-borneol	(+)-Bor	464–43–7	≥ 97%	1 017	1 017	–	1 017	1 017	1 017		25
	C	(–)-myrtenal	Mtal	57526–63–3	≥ 98%	1 236	1 236	1 236	–	1 236	1 236	–	30
		(–)-verbenone	Vn	1196–01–6	≥ 99%	1 573	1 573	1 573	–	1 573	1 573	–	40
		α-terpineol	αT	10482–56–1	≥ 98%	14 401	14 401	14 401	–	14 401	14 401	–	350
		geranyl acetone	GA	3796–70–1	≥ 98%	1 623	1 623	1 623	–	1 623	1 623	–	40
Compounds produced by host spruce tree (*Picea abie*s L.)	D	(±)-α-pinene	αP	80–56–8	≥ 98%	137 280	137 280	137 280	137 280	–	137 280	–	33 100
		(–)-β-pinene	βP	18172–67–3	≥ 99%	138 464	138 464	138 464	138 464	–	138 464	–	33 400
		camphene	Cam	79–92–5	≥ 95%	7 578	7 578	7 578	7 578	–	7 578	–	1 625
		terpinolene	Terp	586–62–9	≥ 90%	5 166	5 166	5 166	5 166	–	5 166	–	1 250
	E	α-terpinene	αTerp	99–86–5	≥ 94%	5 259	5 259	5 259	5 259	5 259	–	–	125
		γ-terpinene	γT	99–85–4	≥ 98.5%	3 928	3 928	3 928	3 928	3 928	–	–	95
		(–)-limonene	(–)-Lim	5989–54–8	≥ 92%	10 093	10 093	10 093	10 093	10 093	–	–	2 435
		(+)-limonene	(+)-Lim	5989–27–5	≥ 98%	10 093	10 093	10 093	10 093	10 093	–	–	2 435

In volumes of 2 mL of heptane, we formulated synthetic blends of the compounds, which were concentrated to correspond the release rates of headspace samples based on Sousa et al. 2023a (for more details about calculations and amount of compounds used see Table 4 in the Appendix or Table 1 in the main text). The synthetic blends were then released for 48-h through "wick-dispensers" that consisted of 5 cm × 1.5 mm Teflon tubing, lined with cotton yarn wick, inserted through a hole drilled in the screw top of a 4–mL glass vial and attached by a wire to the middle of the sticky trap described below (Lejfalk and Birgersson 1997).

2.2 Experiment 1: Subtractive assay on groups of odor compounds

To explore the response of *Medetera* spp. and *I. typographus* to different groups of compounds, a subtractive trapping assay was performed. We compared the activity of the full blend (comprising all five groups ABCDE) to blends in which one group (A, B, C, D, or E) was lacking, i.e., subtracted. Accordingly, six synthetic blends of compounds were prepared as combinations of the different groups (A-E): the full blend (ABCDE) and five subtractive blends prepared by leaving out the bark beetle compounds representing group A (blend BCDE), the microbial compounds representing group B (blend ACDE) or group C (blend ABDE), or the tree compounds representing group D (blend ABCE) or group E (blend ABCD) (Table 1). A control which only contained solvent (heptane) was also included in the assay.

The blends were assessed under field conditions in the period May–July 2021 in four locations at three different forest sites (site 1 (57.151°N, 14.815°E); site 2 (57.176°N, 14.799°E); site 3 (57.164°N, 14.755°E)) located in Asa, Småland province, Southern Sweden. All sites were mixed forest dominated by Norway spruce (*Picea abies* (L.) Karst.) and Scots Pine (*Pinus sylvestris* L) that were over 30 years old. In addition, all three sites were affected by continuous spruce bark beetle outbreaks.

At site 1, two identical sets (I and II) of seven traps were placed at locations approximately 20 m apart. In the first location (set I), the traps were placed in a clearcut area and were sun-exposed. In the second location (set II), the traps were more protected from the sun due to the high density of surrounding trees. At sites 2 and 3, only one set of seven traps was used, at one location each. The locations of these two sites (2 and 3) were similar in terms of low sun exposure. However, site 2 was located near a small lake and was slightly more wind-exposed than sites 1 and 3.

At each location, the seven traps were set in two or three rows (2:3:2 in both locations at site 1 and site 2, 4:3 at site 3). Distance between the traps was approximately 7 m. The trap type used in all cases was a sticky trap made from black standard Norwegian drainpipe (∅ 15 cm × 150 cm, type N79) suspended vertically on a wooden stick at around 30–40 cm above the ground and covered with transparent polyethylene plastic (thickness 50 µm) (Rajapack, Gothenburg, Sweden) that we coated with sticky glue (Sticky-Trap Glue, Marjoman Distribution, Spain). Similarly at the four sampling locations, each of the seven traps was baited with a different synthetic blend (ABCDE, BCDE, ACDE, ABDE, ABCE, ABCD, or heptane control).

To avoid potential positional effects, the position of the synthetic blends at the four sampling locations was rotated six times using a 7 × 7 Latin square design (as illustrated in Table 5 in the Appendix). Within this design, each synthetic blend was strategically positioned once in every row (test round) and column (trap position) within the experimental location. This strategic design allowed a comprehensive evaluation of the potential impact of position on the observed outcomes. Synthetic blends were attached in the center of the traps with a wire and replaced at the end of each test round.

Using this approach, each synthetic blend was tested seven times at the four locations (resulting in a total of 28 replicates per synthetic blend). Each test round lasted for a 48-h assay period. For additional context, information regarding experimental dates and specific weather conditions can be found in Table 6 in the Appendix.

At the end of each test round (48-h assay), the numbers of *Medetera* spp. adult flies and *I. typographus* specimens per sticky trap and synthetic blend were counted and transferred to vials with 76% ethanol and transported to the laboratory for species identification. The sticky plastics of each trap were replaced for the next test round.

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). When possible and needed, representative identified specimens (morphotypes) were compared with specimens from a reference insect collection (private collection of M. Pollet).

To verify the presence of the bark beetles at each location, one additional sticky trap baited with synthetic *I. typographus* aggregation pheromone (PHEROPRAX®, BASF, Limburgerhof, Germany) was placed at around 7 m from the other traps during all test rounds (data not shown). The position of this trap with synthetic pheromone remained the same throughout the test runs. The trap with synthetic pheromone was only used in the experiment 1 in order to confirm that bark beetles were present during the field trial.

2.3 Experiment 2: Subtractive assay on odor compounds within specific groups

In this experiment, three different arrangements of compounds (arrangements 1, 2, and 3) were tested in a 2^{4-1} partial experimental factorial design (Montgomery 2001). In the partial factorial design, a fraction of the total combinations can be selected for experimentation, focusing on specific factors or interactions of interest. This design reduces the number of experimental runs compared to the full factorial design. The factors excluded from the design are typically those believed to have a minimal impact or which are of less interest in the context of the research question.

Compounds cV, Vn, and αP were selected as the factors of interest in the analysis of combinatorial effects as they had already been reported to have either an attractive effect (cV and αP) or a repellent effect (Vn) on *Medetera* spp. (Fitzgerald and Nagel 1972; Bickel 1985; Hulcr et al. 2005).

Arrangement 1 tested the subtractive effects of the bark beetle-produced compounds MB, cV, tV, and Id and the combinatory effect between cV:MB, cV:tV, and cV:Id. Arrangement 2 tested the subtractive effects of compounds produced primarily by symbiotic microorganisms, i.e., Mtal, Vn, αT, and GA, and the combinatorial effect between Vn:Mtal, Vn:αT, and Vn:GA. Arrangement 3 tested the main effects of the tree-produced compounds αP, βP, Cam, and Terp, and the combinatorial effect between αP:βP, αP:Cam, and αP:Terp. The compound tV was also added in arrangements 2 and 3 (Fig. 3).

Compounds from groups B and E were kept constant in the factorial design, since in the previous experiment removing these groups of compounds led to reduced attraction of *Medetera* flies, suggesting the presence of attractive compounds in these two groups.

For each arrangement, eight different synthetic blends were prepared and tested simultaneously (Fig. 3). The concentrations used in the compounds, the type and spacing of sticky traps, and the experiment duration were similar to those in experiment 1.

All three arrangements were tested in bark beetle affected mixed forests dominated mainly by Norway spruce and Scots Pine that were over 30 years old, between May and July 2022. Arrangement 1 was tested at one site (57.650°N, 13.960°E) located at Jönköping, while arrangements 2 and 3 were tested at two sites (57.151°N, 14.815°E; 57.150°N, 14.765°E) located at Asa (as above). The number of replicates was six for arrangement 1 and 16 for each arrangement 2 and 3. Due to bad weather conditions, the Latin square design was not completed for arrangement 1, i.e., only six test rounds instead of eight could be performed. The number of trapped bark beetles and *Medetera* flies was counted after each round. Experimental dates and details about weather conditions can be found in Table 6 in the Appendix.

2.4 Statistical analysis

All statistical analyses were performed in R, with Rstudio (version 4.0.0) (R Core Team 2020) as the interface. For both experiments, the number of *Medetera* adult flies and *I. typographus* beetles collected by each trap was converted to relative percentage calculated from the original counts obtained with each synthetic blend and divided by the sum of counts from all synthetic blends used at the same test round. In experiment 1, a general linear model (GLM) including as factors synthetic blend, time, site, and trap position nested within site was applied using the package *car* (Fox et al. 2012). Pairwise comparisons between control and synthetic blends were made using post hoc *Dunnett's* test from the package *emmeans* (Russell 2019).

In experiment 2, a GLM was also used to determine differences between relative percentage of insects trapped for each synthetic blend in a specific arrangement (1–3). In the model estimation, binary variables for the presence/absence of the synthetic compounds were constructed. The distribution of model residuals in experiments 1 and 2 was checked using *qqnorm* and *qqline* (Becker et al. 1988).

Raw data collected from both experiments can be found in Sousa et al. 2024.

3 Results

3.1 Experiment 1: Subtractive assay on groups of odor compounds

3.1.1 Synthetic blends

Pairwise comparisons between synthetic blends and the control revealed that adult *Medetera* flies were not significantly more attracted to blends BCDE (*Dunnett's*, $t=1.85$; $P=0.27$), ABCD (*Dunnett's*, $t=1.20$; $P=0.65$), or ACDE (*Dunnett's*, $t=2.11$; $P=0.16$) than to the control trap baited with the solvent (Fig. 1A, Table 1). The total

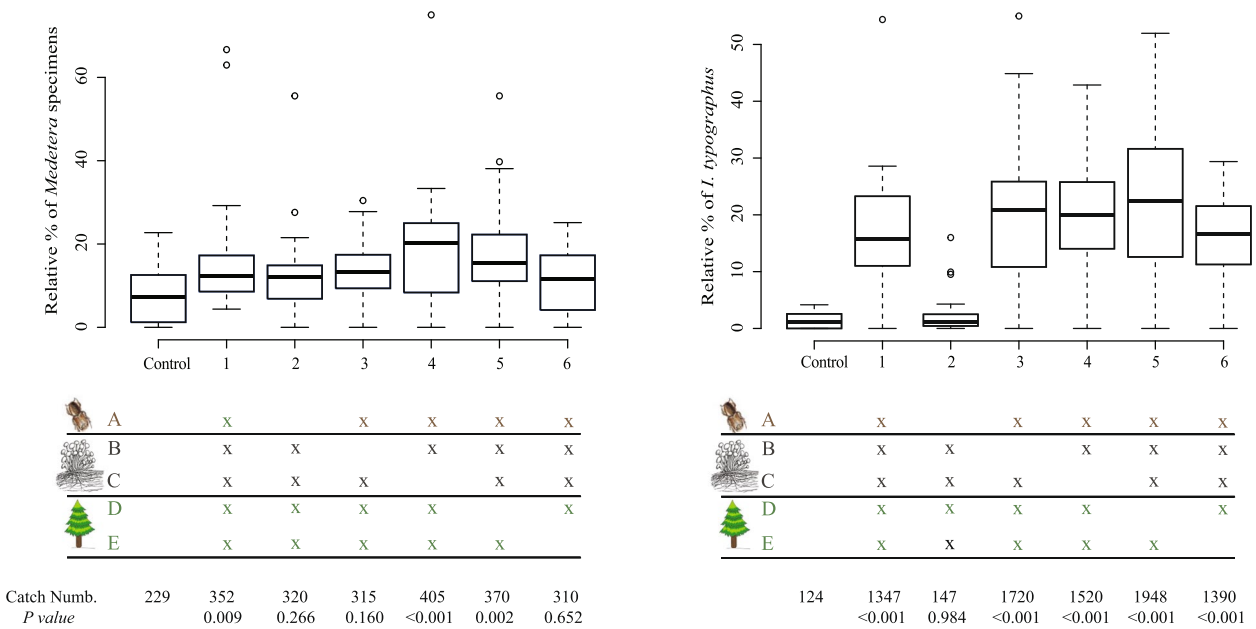


Fig. 1 Relative percentage (%) of trapped **A** *Medetera* flies and **B** *Ips typographus* beetles in the first subtractive assay. Synthetic compounds emitted by bark beetle-infested Norway spruce (*Picea abies*) were divided into five different groups (A–E), where group A contained the bark beetle 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 (number 1 in the figure) contained all compounds from the five groups (ABCDE), while blends numbers 2–6 contained only compounds from four of the groups. The X denotes presence of the compounds from a specific group in each blend. 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 test round and site. *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 ($F = 3.1$; $P < 0.05$)

number of flies collected with these three blends was 320, 310, and 315, respectively, while the control yielded 229 (Fig. 1A). In contrast, ABDE (*Dunnett's*, $t = 4.14$; $P = 0.0003$), ABCE (*Dunnett's*, $t = 3.63$; $P = 0.002$), and ABCDE (*Dunnett's*, $t = 3.18$; $P = 0.009$) were significantly more attractive than the control. The total number of *Medetera* adult flies trapped by these three blends was 405, 370, and 352, respectively (Fig. 1A).

Significant differences were also found in terms of the number of *I. typographus* specimens attracted by the different synthetic blends compared with the control. Blend BCDE (*Dunnett's*, $t = 0.41$; $P = 0.98$), which lacked compounds produced by bark beetles, was the least attractive and trapped a similar number of bark beetles as the solvent control (147 and 124, respectively) (Fig. 1B). Blends ACDE (*Dunnett's*, $t = 7.47$; $P < 0.001$), ABDE (*Dunnett's*, $t = 7.51$; $P < 0.001$), ABCE (*Dunnett's*, $t = 8.56$; $P < 0.001$), ABCD (*Dunnett's*, $t = 6.04$; $P < 0.001$), and ABCDE (*Dunnett's*, $t = 6.29$; $P < 0.001$) trapped significantly more bark beetles than the control trap (Fig. 1B). In total, more than 7000 bark beetles were collected by the synthetic blends. The highest numbers of bark beetles were caught with blends ABCE and ACDE (1948 and 1720 beetles, respectively).

3.1.2 Test rounds and sites

The total number of trapped *Medetera* flies and *I. typographus* differed significantly over the time of the seven test rounds ($F = 30.6$; df = 6,155; $P < 0.001$ and $F = 13.2$; df = 6,155; $P < 0.001$, respectively) (Fig. 2A, B). The total number of trapped *Medetera* flies was higher in the second and third test rounds (between June 8–10 and 17–19, respectively) and again at the seventh, i.e., last, round (July 20–22) (Fig. 2A). Trap yields of *I. typographus* showed a different pattern, with significantly higher numbers for the first test round (30 May–1 June), and further peaks for the fourth and sixth rounds (June 19–21 and July 14–16, respectively) (Fig. 2B).

At site 1, the density of trapped *Medetera* spp. differed significantly between sets I and II ($F = 34.5$; df = 6,18; $P < 0.001$) (Fig. 2C). At site 1, the total number

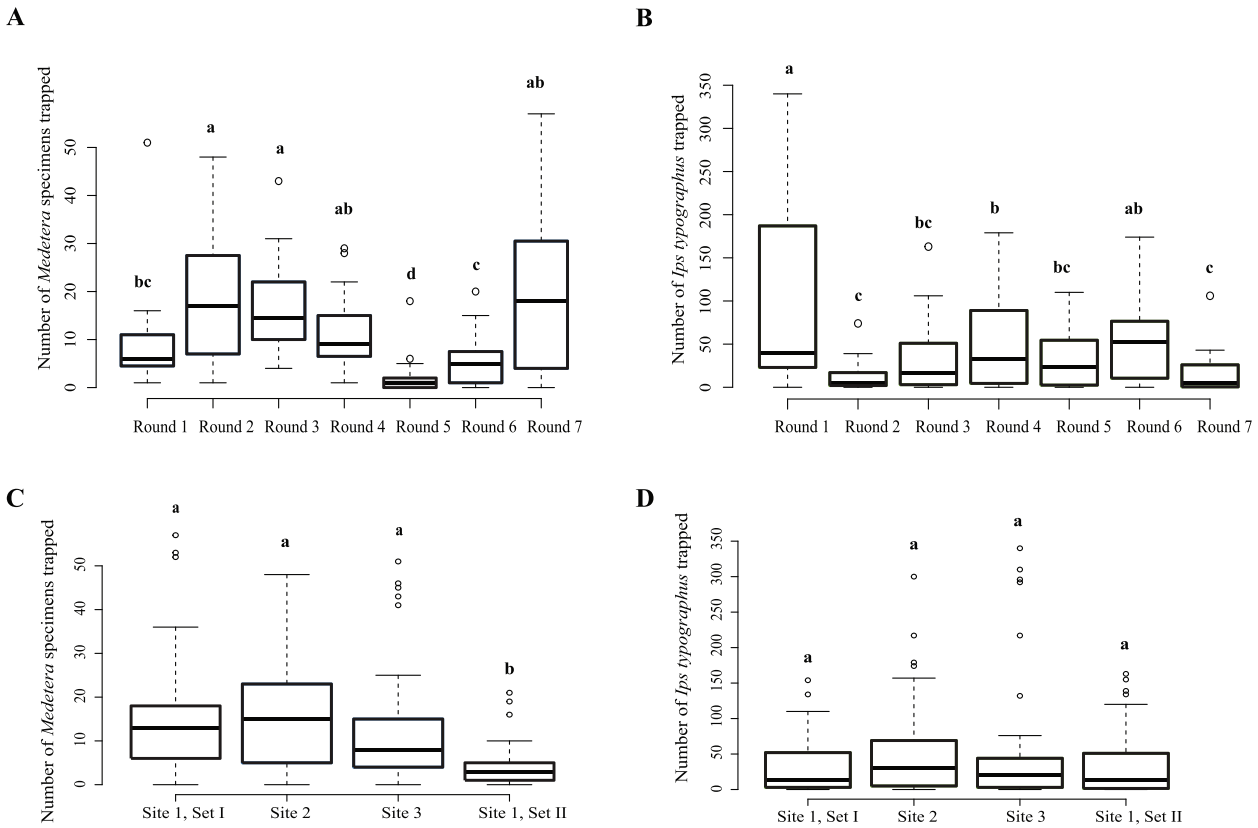


Fig. 2 Total numbers of adult *Medetera* specimens or *Ips typographus* beetles collected in the first subtractive assay. **A**, **B** Specimens trapped at different test rounds (1–7) from mid-May until late July, 2021 based on combined results for all synthetic blends and locations. **C**, **D** Specimens trapped at four different locations (set I and set II at site 1, site 2, site 3), based on combined results for all synthetic blends and test rounds. Different lowercase letters (a, b, c) on top of each box indicate significant differences in total number of specimens found (post-hoc Tukey test following analysis of variance, $P \leq 0.05$)

of flies trapped in set II (191 specimens) was much lower than in set I (756 specimens). In contrast, the total number of *I. typographus* collected did not differ significantly between sites or sets (Fig. 2D).

3.1.3 Species of genus Medetera collected in traps containing the synthetic blends

From the 2301 specimens of *Medetera* adult flies captured in traps containing the synthetic blends over the seven test rounds, a representative sample of 871 specimens, mainly from the second, third, and seventh rounds, were morphologically identified. These comprised 16 different *Medetera* species with *M. signaticornis* ($n=422$), *M. infumata* Loew, 1857 ($n=152$), *M. prjachinae* Negrobov, 1974 ($n=137$), *M. setiventris* Thuneberg, 1955 ($n=78$), *M. excellens* Frey, 1909 ($n=33$), and *M. ambigua* Zetterstedt, 1843 ($n=29$) being the most common species (Table 2). Individuals from these species were found in traps, irrespective of the synthetic blend used. However, *M. signaticornis* was always the most abundant species, regardless of the synthetic blend tested.

In general, the number of fly females trapped was high compared with the number of males. A clear exception to this was found for *M. infumata* for which males were more abundant in traps than females (Table 2). The total number of specimens identified as *M. infumata* trapped during the second and third rounds was much higher than in the seventh (last) round. Similarly, the total number of specimens identified as *M. prjachinae* was highest during the third round, while the number of *M. signaticornis* was highest during the seventh round.

3.2 Experiment 2: subtractive assay on odor compounds within specific groups

In arrangement 1, the strength of attraction of *Medetera* flies differed in response to the different blends ($F=5.4$; df = 1,26; $P<0.001$) (Fig. 3a). In general, flies were more

Table 2 Number of specimens of different *Medetera* species collected on sticky traps baited with six different synthetic blends of odor compounds (designated ABCDE, BCDE, ACDE, ABDE, ABCE, ABCD) and a solvent control during the second, third, and seventh test rounds of experiment 1. F = females, M = males, NI = sex unknown

Species	full (ABCDE)	BCDE	ACDE	ABDE	ABCE	ABCD	control	Total (sex)
Medetera abstrusa Thuneberg, 1955		2		1	3	2		8 (1F; 7 M)
Medetera acanthura Negrobov & Thuneberg, 1970		1	1	1		2	1	6 M
Medetera adjaniae Gosseries, 1988							1	1 M
Medetera ambigua Zetterstedt, 1843	6	2	5	10	1	3	2	29 (18F; 10 M; 1NI)
Medetera apicalis Zetterstedt, 1843						1		1 M
Medetera excellens Frey, 1909	2	8	4	5	3	3	3	33 (29F; 3 M; 1NI)
Medetera fumida Negrobov, 1967		3	1			2	1	7 M
Medetera infumata Loew, 1857	10	16	21	21	29	18	27	142 (38F; 103 M; 1NI)
Medetera melancholica Lundbeck, 1912		1	1	1				3 (2F; 1 M)
Medetera nitida Macquart, 1834				1	1	1	1	4 (2F; 2 M)
Medetera obscura Zetterstedt, 1838			1				1	2F
Medetera pinicola Kowarz, 1877	2		1	3	1	3	2	12 (11F; 1 M)
Medetera prjachinae Negrobov, 1974	22	18	24	23	14	18	18	137 (72F; 64 M; 1 NI)
Medetera setiventris Thuneberg, 1955	14	4	14	13	14	13	6	78 (72F; 6 M)
Medetera signaticornis Loew, 1857	34	67	60	67	67	81	46	422 (267F; 149 M; 6NI)
Medetera tristis Zetterstedt, 1838		1						1 M
Total number of *Medetera* identified	100	136	151	162	140	156	121	871
Total number of *Medetera* collected	352	320	315	405	370	310	229	2 301

attracted ($F=25.3$; df = 1,26; $P<0.0001$) to the blends containing cV compared to the rest of the blends tested in this arrangement (Table 3, arr.1; Fig. 3a; blends 1, 2, 5, 6). We found that the combination between cV and Id ($F=5.08$; df = 1,26; $P=0.03$) (Table 3, arr.1) or between cV and tV ($F=5.25$; df = 1,26; $P=0.03$) (Table 3; arr.1) significantly influenced the number of *Medetera* trapped. The effect of Id alone was also close to significant ($F=4.17$; df = 1,26; $P=0.05$) (Table 3, arr.1). However, no effect of individual tV ($F=0.09$; df = 1,26; $P=0.77$) or MB ($F=0.65$; df = 1,26; $P=0.43$) was observed (Table 3, arr.1).

The bark beetle *I. typographus* also showed significantly different responses to the different blends tested in arrangement 1 ($F=3.86$; df = 1,26; $P<0.01$) (Fig. 3a). In general, blends with cV attracted more bark beetles ($F=19.7$; df = 1,26; $P<0.001$) than blends without this compound (Table 3, arr.1; Fig. 3a, blends 1, 2, 5, 6). The attractive effect of MB was close to significant ($F=3.6$; df = 1,26; $P=0.07$) (Table 3, arr.1). However, there was no clear effect seen for tV and Id, and no significant effect was observed for the combination of cV:MB, cV:tV, or cV:Id (Table 3, arr.1).

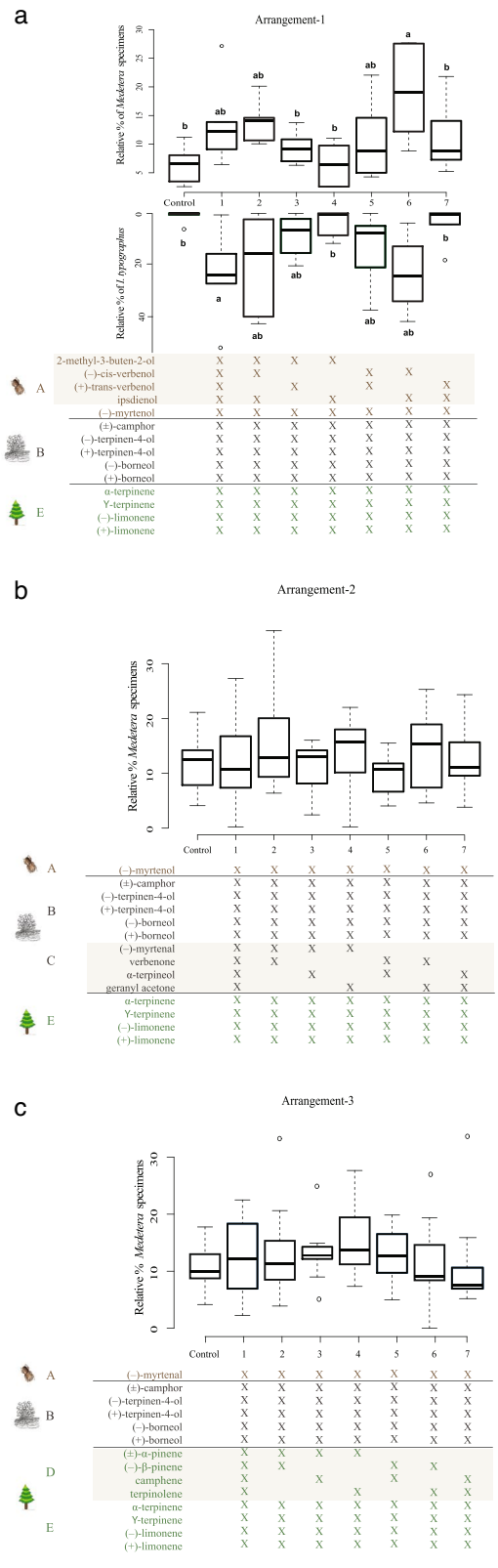
In arrangement 2, no significant differences were seen between the different blends (Fig. 3b). The compounds Mtal, Vn, αT, and GA, which are primarily produced by microorganisms, had a no effect ($P>0.05$) in attraction of *Medetera* adult flies (Table 3, arr.2). However, the combinatory effect of Vn and αT was close to significant ($F=3.39$; df = 1,66, $P=0.07$) (Table 3, arr2), indicating that the combination of these two compounds might have a negative effect on the number of flies collected. Similarly, in arrangement 3, no significant differences were found between the different blends (Fig. 3c). Compounds produced by the host tree (αP, βP, Cam, Terp) had no effect ($P>0.05$) in attraction of *Medetera* flies and showed no combinatory effects (Table 3, arr.3).

Furthermore, very few *I. typographus* specimens were collected in the traps containing blends from arrangements 2 and 3 (Table 7 in the Appendix). Neither of these two groups contained either of the two aggregation pheromone components.

Overall, the 12-component blend (Fig. 3a, blend 6) containing the odor compounds (−)-cis-verbenol, ipsdienol, (−)-myrtenol, (−)-limonene, (+)-limonene, α-terpinene, γ-terpinene, (±)-camphor, (−)-terpinen-4-ol, (+)-terpinen-4-ol, (−)-borneol, and (+)-borneol showed the most distinct attraction of *Medetera* spp. specimens, differing significantly to the solvent control.

4 Discussion

Medetera flies are among the most important natural enemies of the spruce infesting bark beetle *I. typographus*. However, we lack knowledge about the ecology of *Medetera* species including their presence, abundance,



◄ **Fig. 3** Relative percentage (%) of trapped *Medetera* flies and *Ips typographus* beetles in three arrangements of different synthetic blends and solvent controls tested in the second subtractive assay using the partial factorial design (2^{4-1}). Three different arrangements (1, 2, and 3) of seven synthetic blends (1–7) were established. The X denotes presence of the compounds from a specific group in each blend. The control contained only the solvent heptane. The relative percentage of trapped *Medetera* and *Ips typographus*, respectively was calculated for the individual treatments relative to the overall number of trapped specimens at the same test round and site. A general linear model (GML) followed by analysis of variance (ANOVA) was used to study significant differences between the relative percentage of insects trapped by each synthetic blend based on the presence or absence of blend components. Different lowercase letters (a, b, c) indicate significant differences ($P \leq 0.05$) between treatments (post hoc Tukey test following ANOVA). In arrangements 2 and 3, the data for *I. typographus* was not included due to the very low number of beetles collected by these synthetic blends

distribution, and trophic interactions in forests. Following up on our previous study of volatiles collected from bark beetle infested spruce trees and their ability to induce antennal and behavioral responses in *Medetera* flies (Sousa et al. 2023a), we designed two trapping experiments for a systematic investigation of behavioral responses to synthetic blends of the earlier identified volatiles.

4.1 Behavioral responses of *Medetera* spp. to synthetic blends and effects of compounds

Compounds previously identified from bark beetle infested spruce trees had been categorized as being produced by the spruce trees, the *I. typographus* beetles or associated microorganisms (Sousa et al. 2023a). In the present study, adult *Medetera* flies were less attracted to the synthetic blends that lacked tree-produced compounds such as (–)-limonene, (+)-limonene, α-terpinene and γ-terpinene (represented in group E), microbial-produced compounds such as (±)-camphor, (–)-terpinen-4-ol, (+)-terpinen-4-ol, (–)-borneol and (+)-borneol (representing group B) and bark beetle-produced compounds 2-methyl-3-buten-2-ol, (–)-*cis*-verbenol, (+)-*trans*-verbenol, (–)-myrtenol, and ipsdienol (represented in group A). Accordingly, behaviorally active compounds involved in the attraction of *Medetera* flies to bark beetle infested trees originate from all the three categories.

Bark beetles are known to emit pheromones to communicate with each other and coordinate various behaviors, such as aggregation and/or mating. Previous studies have demonstrated that several *Medetera* spp.

Table 3 Summary of the effects of synthetic compounds on trapped *Medetera* flies and *Ips typographus* beetles in the different arrangements (1–3) of experiment 2

Arrangement 1 (df = 26)	*Medetera* specimens	*I. typographus*	Arrangement 2 (df = 66)	*Medetera* specimens	Arrangement 3 (df = 66)	*Medetera* specimens
MB	$F=0.65$; $P=0.43$	$F=3.6$; $P=0.07$	αT	$F=0.65$; $P=0.42$	αP	$F=0.73$; $P=0.39$
cV	$F=25.3$; $P<0.001$	$F=19.7$; $P<0.001$	Mtal	$F=1.42$; $P=0.24$	βP	$F<0.01$; $P=0.96$
tV	$F=0.09$; $P=0.77$	$F=0.09$; $P=0.75$	Vn	$F=0.51$; $P=0.48$	Cam	$F<0.01$; $P=0.92$
Id	$F=4.17$; $P=0.05$	$F=1.8$; $P=0.18$	GA	$F=0.41$; $P=0.53$	Terp	$F=0.14$; $P=0.71$
cV:MB	$F=0.08$; $P=0.77$	$F=0.02$; $P=0.88$	Vn: αT	$F=3.3$; $P=0.07$	αP:βP	$F=2.56$; $P=0.11$
cV:tV	$F=5.25$; $P=0.03$	$F=0.02$; $P=0.87$	Vn:Mtal	$F=1.19$; $P=0.28$	αP:Cam	$F=1.41$; $P=0.24$
cV:Id	$F=5.08$; $P=0.03$	$F=2.37$; $P=0.13$	Vn:GA	$F=1.16$; $P=0.29$	αP:Terp	$F<0.01$; $P=0.95$

Arrangement 1 tested the effects of the bark beetle-produced compounds 2-methyl-3-buten-2-ol (MB), (−)-cis-verbenol (cV), (+)-trans-verbenol (tV), and (±)-ipsdienol (Id), and the combination between cV:MB, cV:tV and cV:Id. Arrangement 2 tested the effects of compounds produced primarily by symbiotic microorganisms (−)-myrtenal (Mtal), verbenone (Vn), α-terpineol (αT), and geranyl acetone (GA), and the combination between Vn:αT, Vn:Mtal and Vn:GA. Arrangement 3 tested the effects of the tree-produced compounds (±)-α-pinene (αP), β-pinene (βP), camphene (Cam), and terpinolene (Terp), and the combination between αP:βP; αP:Cam and αP:Terp. Df-degrees of freedom, *F*-values indicate variation between samples, $P<0.05$ indicates a significant difference between treatments. In arrangements 2 and 3, the data for *I. typographus* was not included due to the very low number of beetles collected by these synthetic blends

are attracted to bark beetle pheromones. For example, in studies in North America, *M. bistriata* Parent, 1929 is attracted to frontalin, a key pheromone component produced by *Dendroctonus frontalis* Zimmermann, 1868 and *D. brevicomis* LeConte, 1876 bark beetles (Vité and Pitman 1969; Williamson 1971). Studies in Europe have shown that *M. melancholica* Lundbeck, 1912 is attracted to a combination of both components of the aggregation pheromone of *I. typographus* (2-methyl-3-buten-2-ol and (−)-*cis*-verbenol) and that *M. setiventris* is attracted to (−)-*cis*-verbenol alone (Hulcr et al. 2005). In another study in Europe, Hulcr et al. (2006) showed that the number of *M. setiventris* attracted to traps increased when (−)-*cis*-verbenol was combined with ipsdienol. Similarly, in the present study, it was found that *Medetera* spp. were more attracted to blends containing (−)-*cis*-verbenol and that the number of trapped flies increased when (−)-*cis*-verbenol was combined with ipsdienol, or with (+)-*trans*-verbenol. However, in this study no significant effect of 2-methyl-3-buten-2-ol was observed, while previously, the antennae of *M. signaticornis* males and females were found to detect (−)-*cis*-verbenol and (+)-*trans*-verbenol (Sousa et. al. 2023a). Also, the oligophagous predator *Thanasimus formicarius* Linnaeus, 1758 is known to detect several *I. typographus*-produced compounds (2-methyl-3-buten-2-ol, (−)-*cis*-verbenol, (+)-*trans*-verbenol, ipsdienol) but seems to be most attracted to ipsdienol and (−)-*cis*-verbenol (Bakke and Kvamme 1981; Tømmerås 1985; Schroeder 2003).

Furthermore, it has previously been shown that (±)-camphor (a component of group B) combined with the *I. typographus* aggregation pheromone significantly increases the number of trapped *M. setiventris* but has no effect on *M. melancholica* (Hulcr et al. 2005). These findings by Hulcr et al. (2005), together with our data, indicate that different *Medetera* species may use different chemical cues to detect bark beetle-infested trees.

Also, attraction of *M. signaticornis* and *M. setiventris* adults to limonene (used in group E) has been reported previously (Rudinsky et al. 1971; Hulcr et al. 2005). According to Hulcr et al. (2005), limonene alone is not sufficient to attract *M. setiventris*, while a combination of limonene with the *I. typographus* aggregation pheromone and (±)-α-pinene increased the number of flies trapped. In our study, *Medetera* flies were significantly more attracted to the blend that lacked (±)-α-pinene but also (−)-β-pinene, camphene, and terpinolene (represented in group D) compared with the control. These results suggest that these compounds might not play a significant role in the finding of bark beetle infested spruce trees. Since all four compounds are reported to be active on *M. signaticornis* antennae, they may have alternative behavioral functions (Sousa et al. 2023a). For example, (±)-α-pinene has been reported to act as an oviposition stimulus for gravid *M. aldrichii* females and to guide newly emerged larvae from oviposition sites towards prey galleries (Fitzgerald and Nagel 1972).

The most attractive blend was lacking a group of oxygenated monoterpenes such as (−)-myrtenal, α-terpineol, (−)-verbenone, and geranyl acetone (represented in group C) primarily produced by bark

beetle-associated microorganisms. In this group, the compound (–)-verbenone is known to be produced in higher amounts in late stages of bark beetle attack, through conversion of (–)-*cis*-verbenol (Leufvén et al. 1984; Frühbrodt et al. 2023). The compound (–)-verbenone has an anti-aggregation effect on *I. typographus*, which counteracts the attraction of bark beetles to their aggregation pheromone (Schlyter et al. 1989; Lindgren and Miller 2002b) and has been shown to have a repellent effect on *T. formicarius* (Etxebeste and Pajares 2011; Lindgren and Miller 2002a). In an earlier study, the density of *M. bistriata* around bark beetle-infested fallen trees was seen to be significantly reduced by the anti-aggregation pheromone 3-methyl-2-cyclohexen-1-one of Douglas fir beetle (*Dendroctonus pseudotsugae* Hopkins, 1905) (Furniss et al. 1979). Accordingly, absence of beetle anti-aggregation compound (–)-verbenone from synthetic blends might have increased the number of *Medetera* spp. trapped.

In this study, we analyzed different synthetic blends that were formulated from a selection of 22 compounds. Using a partial factorial design, we were able to define a 12-compound synthetic blend that induced significant attraction to *Medetera* flies. Despite this achievement, general limitations of a partial factorial test design should be considered, for example, some main effects and interactions between compounds can be confounded, meaning they remain indistinguishable from one another. This can cause difficulties to separate the effects of individual factors. Moreover, the reduced number of runs results in lower statistical power, which can limit the detection of significant effects, especially if the true effects are small. Furthermore, not all combinations of factor levels are tested, which can result in incomplete information about the studied system. For more information on partial factorial design, we refer to Montgomery (2001). Overall, we see the partial factorial design as a useful tool that allows strategic planning and management of experiments and results when practical limitations (time and funding) restrict full factorial testing.

4.2 Fly species identities, abundance, sex, and temporal variation

Although morphological identification of *Medetera* species is difficult and often requires microscopic examination of genitals (Pollet et al. 2011, 2022), we identified a significant subset of trapped flies (800 specimens) to species level. The three *Medetera* species that were collected in highest numbers, i.e., *M. signaticornis*, *M. infumata*, and *M. prjachinae*, have all been found previously on *I. typographus*-infested Norway spruce trees (Hedgren and Schroeder 2004; Wermelinger et al. 2012; Sousa et al. 2023b).

For most species, females were more abundant on the sticky traps than males, indicating that compounds released from beetle-infested trees likely help females to find suitable oviposition sites. However, a largely overlapping set of compounds is probably also used by the males to find mating sites and correspondingly, more males *M. infumata* were caught than females. According to Hopping (1947), aggregation of *Medetera* on tree trunks facilitates mating and, at the mating sites, males were reported to be more abundant than females. Extensive collections of *M. veles* Loew, 1861 and *M. vittata* Van Duzee, 1919, taken from one single tree, revealed a male to female ratio of 2:1 or 3:1 (Bickel 1985). Similar male–female ratios on tree trunks have also been found for other *Medetera* species (Runyon 2020).

Our results also showed that the number of specimens trapped changed slightly over time, being highest immediately after the two seasonal peaks in *I. typographus*. This variation might be related to species-specific seasonal population development and behavioral activity of *Medetera* species (Beaver 1966; James 2023) and may also have been influenced by changing local weather conditions during the experimental period. In Sweden, differences in emergence patterns for several *Medetera* spp. have already been reported e.g., *M. signaticornis* (most common species) was observed to be the earliest, followed by *M. pinicola* and *M. ambigua* (James 2023). Furthermore, in another study adults of *M. signaticornis* have also been reported to be more abundant during summer than in spring and autumn (Wermelinger et al. 2012). However, detailed information on the flight activity patterns of *Medetera* spp. is still scarce.

4.3 Response of *I. typographus* to synthetic blends, effects, and interactions between compounds

Primarily, we were interested in understanding the chemical information used by *Medetera* flies to find *I. typographus* as their prey and to develop a synthetic attractant. In this context, we were also interested if identical chemical compounds are used by predator and prey. From an applied point of view, co-attraction of pest and natural enemies is of relevance and limits the use of synthetic attractants when aiming at behavioral manipulation of natural enemies to antagonize pests (Sant'Ana et al. 1997; Simpson et al. 2011; Salamanca

et al. 2019). We found that several of the tested synthetic blends attracted *I. typographus*.

The number of *I. typographus* trapped was lower for the blend that lacked the bark beetle-produced compounds. This was expected, since both (–)-*cis*-verbenol and 2-methyl-3-buten-2-ol are essential for attraction and guidance of *I. typographus* bark beetle males and females to host trees (Schlyter et al. 1987; 1989). The aggregation pheromone component (–)-*cis*-verbenol is produced by detoxification of the host terpene (±)-α-pinene and is suggested to act as a long-range attractant directing bark beetles to newly infested host trees. In contrast, the compound 2-methyl-3-buten-2-ol, which can be produced by the beetles de novo, is suggested to promote short-range orientation and landing on the infested host tree (Lanne et al. 1989; Schlyter et al. 1987; Lindström et al. 1989; Birgersson et al. 1984). Previous studies have reported significantly increased catches when small amounts of ipsdienol were added to a combination of 2-methyl-3-buten-2-ol and (–)-*cis*-verbenol, but no effect was observed in relation to (–)-myrtenol (Schlyter et al. 1987). In the present study, there was no positive or negative effect of ipsdienol or (–)-myrtenol seen.

Interestingly, both (–)-*cis*-verbenol and ipsdienol are produced by several bark beetle species e.g., *I. amitinus* Eichhoff, 1872 (Wood 1982; Symonds and Elgar 2004) that are sympatric with *I. typographus* (Wood 1982; Symonds and Elgar 2004). It is thus likely that *Medetera* spp. which respond to (–)-*cis*-verbenol and ipsdienol are able to detect and perhaps prey on sympatric species producing these compounds. Further studies on chemical ecology, species-specific predator–prey relationship, and host range are needed to clarify this possibility.

In experiment 1, all synthetic blends that contained the bark beetle produced compounds collected substantial numbers of *I. typographus*. The blend that lacked host tree-produced compounds from group D ((±)-α-pinene, (–)-β-pinene, camphene, and terpinolene) attracted the highest number of beetles. Host tree monoterpenes have already been reported to influence the response of bark beetles, e.g., (–)-α-pinene can enhance or inhibit the response of *I. typographus* to their aggregation pheromone, depending on the release rates and environmental conditions (Erbilgin and Raffa 2001; Erbilgin et al. 2007; Olenici et al. 2007). Monoterpenes such as (–)-α-pinene are part of the host tree defense mechanisms against herbivores, including bark beetles. While they might attract *I. typographus* in certain situations, these compounds can also play a role in deterring or protecting against infestations.

The abundance of *I. typographus* trapped in the present study changed over time and might depended on beetle emergence, flight activity, and weather conditions. In southern Sweden, *I. typographus* can be univoltine or bivoltine depending on the weather (Jönsson 2007; Jönsson et al. 2009). In most years *I. typographus* only has one generation, but during years with an early and warm spring (i.e., > 20 °C in mid-April) and long warm summer, as in 2006 and 2018, *I. typographus* can produce two true generations. Emergence of the second brood usually happens during late June and July, depending on the timing of the first flight period. However, in most years, the second brood will not emerge during the autumn and will remain under the bark during the winter. Considering this information, and the weather conditions during the year 2021 our results indicate that the first peak with higher numbers of trapped *I. typographus* obtained at the end of May might correspond to the first flight of parental beetles that have overwintered, most probably under the soil (Botterweg 1982). While, the second peak recorded during mid-July most probably corresponds to emergence and flight of the first seasonal brood of bark beetles.

5 Conclusions and future focus

This study provides a foundation for the development of chemical lures facilitating efficient monitoring of *Medetera* flies in the future. Based on a classical chemical–ecological approach of isolation, identification, and physiological testing of biological compounds in a previous study, the subtractive and partial factorial test design of the current study allowed us to reduce the components of a complex attractive chemical blend while maintaining significant trap catches of *Medetera* flies. Our approach of testing a subset of the possible permutations of factors could guide other researchers in designing smart experiments for combinatorial testing of compounds. Taxonomic identification of a subsample of these flies clarified that a community of several species responds to the same blend, with *M. signaticornis* being most abundant. In the tested blends, pheromone compounds of *I. typographus* were necessary to attract *Medetera* flies, suggesting exploitation of beetle signals by their natural enemies. Exploring ecological dynamics of *Medetera* spp. in various forest ecosystems and incorporating semiochemicals and molecular techniques for species identification and monitoring could further refine our understanding of their diversity and behavior and emphasize their role as natural enemies of bark beetles.

Appendix

Table 4 Calculations of the amounts of compounds used in experiment 1 and 2

	Step 1			Step 2				Step 3			Step 4			
	ng release per hour	ng release during 24 hours	µg release during 24 hours	Ideal standard bait as µg per 24h	Density	Ideal standard bait as µL per 24h	Ideal standard bait as µL per 48h	mL solvent in stock batch	ng/µL	µg/µL in stock batch	IDEAL µg/48h	µL in 2mL C_7	µg in 1mL bait = µg/48h	µg in 2mL bait= µg/48h
2-methyl-3-buten-2-ol	6 020	144 479	144	145	0.824	175	351	1.65	144 479	144.5	290	20	1 445	2 890
(–)-*cis*-verbenol	10 075	241 793	242	240	solid	242mg	484mg	1.52	24 000	24.0	480	20	240	480
(+)-*trans*-verbenol	13 319	319 650	320	320	1.000	320	639	1.36	319 650	319.7	640	20	3 197	6 393
(–)-myrtenol	5 152	123 638	124	125	0.954	130	259	1.74	123 638	123.6	250	20	1 236	2 473
ipsdienol	1 630	39 111	39	40	0.900	43	87	1.91	39 111	39.1	80	20	391	782
camphor	6 943	166 638	167	165	0.992	168	336	1.66	166 638	166.6	330	20	1 666	3 333
terpinen-4-ol	10 491	251 782	252	250	0.933	270	540	1.46	251 782	251.8	500	20	2 518	5 036
myrtenal	2 575	61 790	62	60	0.988	63	125	1.87	61 790	61.8	120	20	618	1 236
borneol	4 237	101 686	102	100	1.011	101	201	1.80	101 686	101.7	200	20	1 017	2 034
(–)-verbenone	3 278	78 669	79	80	0.975	81	161	1.84	78 669	78.7	160	20	787	1 573
α-terpineol	30 002	720 045	720	720	0.934	771	1542	0.46	720 045	720.0	1 440	20	7 200	14 401
geranyl acetone	3 381	81 146	81	80	0.873	93	186	1.81	81 146	81.1	160	20	811	1 623
(±)-α-pinene	2 829 706	67 912 946	67 913	68 000	0.858	79 153	neat	none	858 000	858	136 000	160	68 640	137 280
(–)-β-pinene	2 848 245	68 357 874	68 358	68 000	0.865	78 990	neat	none	865 400	865	136 000	160	69 232	138 464
camphene	150 019	3 600 457	3 600	3 600	0.842	4 276	neat	none	842 000	842	7 200	9	3 789	7 578
terpinolene	109 142	2 619 400	2 619	2 620	0.861	3 042	neat	none	861 000	861	5 240	6	2 583	5 166
α-terpinene	10 957	262 970	263	265	0.837	314	628	1.37	262 970	263.0	530	20	2 630	5 259
g-terpinene	8 183	196 392	196	200	0.850	231	462	1.54	196 392	196.4	400	20	1 964	3 928
(±)-limonene	413 344	9 920 268	9 920	10 000	0.841	11 794	neat	none	841 100	841	20 000	24	10 093	20 186

Step 1: Amounts of compounds found to be released from living Norway spruce trees infested with the bark beetle *Ips typographus*; Step 2: Based on Step1 the ideal amount of each compound in µL to be released over 48h from a standard bait was determined; Step 3: 2 mL stock batch of each compound was prepared in heptane (solvent); Step 4: In volumes of 2 mL of heptane, we then formulated synthetic blends of the compounds from each stock batch

Table 5 Latin square design used in the subtractive experiment 1

Site 1. Set-I	Trap position						
Test round	1A:1	1A:2	1A:3	1A:4	1A:5	1A:6	1A:7
1	full (ABCDE)	ABCE	BCDE	ABCD	ACDE	control	ABDE
2	ABDE	ACDE	control	BCDE	full (ABCDE)	ABCE	ABCD
3	ABCD	BCDE	ACDE	full (ABCDE)	ABCE	ABDE	control
4	ABCE	ABCD	ABDE	control	BCDE	full (ABCDE)	ACDE
5	ACDE	full (ABCDE)	ABCE	ABDE	control	ABCD	BCDE
6	BCDE	control	ABCD	ABCE	ABDE	ACDE	full (ABCDE)
7	control	ABDE	full (ABCDE)	ACDE	ABCD	BCDE	ABCE

Site 1. Set-II	Trap position						
Test round	1B:1	1B:2	1B:3	1B:4	1B:5	1B:6	1B:7
1	ABDE	BCDE	full (ABCDE)	control	ABCE	ACDE	ABCD
2	ABCE	ABCD	ABDE	full (ABCDE)	control	BCDE	ACDE
3	full (ABCDE)	ACDE	ABCE	BCDE	ABCD	ABDE	control
4	BCDE	ABCE	control	ABCD	ACDE	full (ABCDE)	ABDE
5	ACDE	control	ABCD	ABDE	full (ABCDE)	ABCE	BCDE
6	control	ABDE	ACDE	ABCE	BCDE	ABCD	full (ABCDE)
7	ABCD	full (ABCDE)	BCDE	ACDE	ABDE	control	ABCE

Site 2	Trap position						
Test round	2A:1	2A:2	2A:3	2A:4	2A:5	2A:6	2A:7
1	control	ACDE	ABCD	BCDE	ABCE	full (ABCDE)	ABDE
2	ABCE	full (ABCDE)	ABDE	control	BCDE	ACDE	ABCD
3	ACDE	control	ABCE	ABCD	full (ABCDE)	ABDE	BCDE
4	ABDE	ABCE	control	ACDE	ABCD	BCDE	full (ABCDE)
5	BCDE	ABDE	ACDE	full (ABCDE)	control	ABCD	ABCE
6	ABCD	BCDE	full (ABCDE)	ABDE	ACDE	ABCE	control
7	full (ABCDE)	ABCD	BCDE	ABCE	ABDE	control	ACDE

Site 3	Trap position						
Test round	3A:1	3A:2	3A:3	3A:4	3A:5	3A:6	3A:7
1	ABDE	full (ABCDE)	ACDE	control	BCDE	ABCD	ABCE
2	ABCD	control	BCDE	full (ABCDE)	ABDE	ABCE	ACDE
3	ACDE	ABDE	ABCE	BCDE	ABCD	full (ABCDE)	control
4	BCDE	ABCE	ABDE	ACDE	full (ABCDE)	control	ABCD
5	ABCE	ABCD	full (ABCDE)	ABDE	control	ACDE	BCDE
6	control	BCDE	ABCD	ABCE	ACDE	ABDE	full (ABCDE)
7	full (ABCDE)	ACDE	control	ABCD	ABCE	BCDE	ABDE

Table 6 Experimental dates and weather conditions. (-) no available information. Data can be accessed from the Asa weather station, Anon (2023)

	Test round (48-h assay)	Dates (start and end of each round)	Temperature (°C) average	Precipitation (mm) average
Experiment 1				
	1	2021-05-30	13.8	0.0
	1	2021-05-31	17.1	0.0
	1	2021-06-01	13.7	0.0
	2	2021-06-08	16.8	0.0
	2	2021-06-09	16.5	0.0
	2	2021-06-10	16.7	0.0
	3	2021-06-17	18.6	0.0
	3	2021-06-18	23.5	0.0
	3,4	2021-06-19	23.8	0.0
	4	2021-06-20	22.9	0.1
	4	2021-06-21	19.8	4.4
	5	2021-07-05	18.9	4.0
	5	2021-07-06	19.3	1.5
	5	2021-07-07	17.2	2.7
	6	2021-07-14	23.1	0.0
	6	2021-07-15	23.8	0.0
	6	2021-07-16	22.3	0.0
	7	2021-07-20	15.7	0.0
	7	2021-07-21	18.0	0.0
	7	2021-07-22	18.6	0.0

	Test round (48-h assay)	Dates (start and end of each round)	Temperature (°C) average	Precipitation (mm) average
Experiment 2				
	1	2022-05-18	10.8	-
	1	2022-05-19	15.4	-
	1	2022-05-20	14.3	-
	2	2022-06-15	14.4	-
	2	2022-06-16	15.5	-
	2,3	2022-06-17	16.8	-
	3	2022-06-18	16.3	-
	3	2022-06-19	12.6	-
	4	2022-07-08	14.8	-
	4	2022-07-09	15.7	-
	4	2022-07-10	13.9	-
	5	2022-07-11	15.3	-
	5	2022-07-12	18.0	-
	5	2022-07-13	18.3	-
	6	2022-07-22	18.5	-
	6	2022-07-23	15.8	-
	6	2022-07-24	16.2	-
	7	2022-07-31	16.1	-
	7	2022-08-01	15.9	-
	7	2022-08-02	15.3	-

Table 7 Total numbers of *Ips typographus* beetles collected in the traps with synthetic blends tested in arrangements 2 and 3 of experiment 2

Blends	Arr.2	Arr.3
1	45	18
2	16	2
3	4	9
4	8	0
5	6	6
6	3	2
7	2	3
Control	0	6

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Availability of data and materials
All data of this study are provided in the results and raw data can be made available on request.

Declarations

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