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Field effects of oxygenated monoterpenes and estragole combined with pheromone on attraction of Ips typographus and its natural enemies

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Introduction: Central European Norway spruce monocultures face *Ips typographus* outbreaks due to decreasing resistance. These beetles use volatile compounds to communicate and select suitable host trees. Spruce trees, beetles, and their symbiotic ophiostomatoid fungi emit oxygenated monoterpenes, including 1,8-cineole, α-terpineol, camphor, carvone, terpinen-4-ol, isopinocamphone, and pinocamphone, and the phenylpropanoid estragole, particularly in the infestation phase. These compounds trigger strong responses in *I. typographus* antennae, motivating our field study.

Objective: This study aimed to assess how adding these compounds to the aggregation pheromone of *Ips typographus* modulates the attraction of this bark beetle and its natural enemies.

Methods: In combination with *I. typographus* pheromone, estragole, 1,8-cineole, (±)-camphor, (−)-carvone, *alpha*-terpineol, (−)-terpinen-4-ol, (+)-pinocamphone, and (+)-isopinocamphone at low, medium, and high doses were tested in pheromone traps at two sites in the Czech Republic.

Results: All 1,8-cineole doses and the high estragole dose acted as anti-attractants for *I. typographus*, whereas all (+)-isopinocamphone doses enhanced their attraction to pheromone. Catches of natural enemies, the Staphylinidae and Pteromalidae, varied by location.

Conclusion: 1,8-cineole, isopinocamphone, and estragole may play vital roles in tritrophic interactions among spruce trees, and *I. typographus* and its natural enemies, and these compounds may be developed into new or enhanced semiochemical-based pest control methods.

KEYWORDS

Eurasian spruce bark beetle, host compounds, Pteromalidae, Staphylinidae, Norway spruce, *Picea abies*, spruce kairomone, pheromone traps

1 Introduction

In Central Europe, Norway spruce (*Picea abies*) (L.) Karst. (Pinales: Pinaceae) has been severely affected by infestations of the spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Curculionidae: Scolytinae), that in the Czech Republic have resulted in timber losses of 5.9 mil. m^3 in 2017 and 26.2 mil. m^3 in 2020 (Hlásny et al., 2022). In outbreak regions, managing bark beetles often involves applying insecticides to *P. abies* trunks or stored timber to eliminate the emerging beetles (Fettig and Hilszczański, 2015). However, the use of pesticides can negatively impact the forest ecosystem, including beneficial bark beetle predator species (Hlásny et al., 2019). The development of alternative, eco-friendly strategies in forestry is a logical progression. One such strategy involves utilizing semiochemicals, compounds that mediate the interactions of beetles with each other and other organisms. These signals enable beetles to locate a mate or host tree by providing intraspecific and interspecific chemical information (Bergström, 2007). Aggregation pheromone components, produced by male beetles after successful colonization (Birgersson et al., 1984; Ramakrishnan et al., 2022), have been employed for monitoring and controlling *I. typographus* populations (Heber et al., 2021). Furthermore, recent research has focused on the management potential of kairomones, compounds originating from both host spruce trees and non-host trees, e.g., broadleaf trees (Zhang and Schlyter, 2004; Jakuš et al., 2022). Bark beetles possess a sophisticated olfactory system that enables them to detect and distinguish the chemical composition and quantity of these odors (Andersson, 2012).

The principal olfactory stimulants for *I. typographus* emitted by *P. abies* are primarily composed of high-abundance monoterpenes such as α-pinene (23–39%), β-pinene (25–58%), β-phellandrene (5–19%), limonene (1.5–4%), myrcene (1.6–3.4%), Δ-3-carene (0.6–1.1%), and camphene (0.2–1.1%; Netherer et al., 2021). However, recent comparative analysis utilizing *I. typographus* antennae as biological detectors (gas chromatography coupled with electroantennography, GC-EAD) has identified several novel compounds present in relatively small amounts but exhibiting high activity with the beetles' antennae (Kalinová et al., 2014; Schiebe et al., 2019). These compounds include oxygenated monoterpenes, 1,8-cineole (eucalyptol), *trans*-4-thujanol (sabinene hydrate), camphor, pinocarvone, pinocamphone, isopinocamphone, terpinen-4-ol, alpha-terpineol, carvone, and phenylpropanoid estragole (4-allylanisole and methyl chavicol). In single-cell electrophysiological studies, researchers identified 24 classes of olfactory sensory neurons (OSN) within olfactory sensillae for *I. typographus* (Hallberg, 1982). Plant odor-responding OSNs exhibit a variety of response specificities from broadly tuned OSNs for host monoterpene hydrocarbons to several highly specific OSN classes responding mainly to oxygenated monoterpenes (1,8-cineole, isopinocamphone, *trans*-4-thujanol, or verbenone; Andersson et al., 2009; Schiebe et al., 2019; Kandasamy et al., 2023).

In Norway spruce, oxygenated monoterpenes are minor compounds (∼1% representation), and their content is influenced by tree health and stress levels (Netherer et al., 2021). The production of oxygenated monoterpenes in trees naturally occurs through the cytochrome P450-catalyzed oxidation of monoterpene hydrocarbons or by cyclization of oxygenated

intermediates (Celedon and Bohlmann, 2019). The release rate of oxygenated monoterpenes, including 1,8-cineol, camphor, pinocarvone, terpinen-4-ol, and α-terpineol, from healthy trees at 24°C ranges from 0.1 to 7 µg/m²/h of stem surface area (Ghimire et al., 2016). In infested trees, these rates increased 10–100 times (Jaakkola et al., 2022), and in cut trees, they increased 10 times (Schiebe et al., 2019).

Bark beetle symbiotic ophiostomatoid fungi generate oxygenated terpenes in laboratory conditions when they are inoculated onto a wood substrate (Kandasamy et al., 2023). In the forest, fungi may assist beetles in colonizing healthy trees by being involved in detoxifying host defense terpenes (Krokene, 2015; Kandasamy et al., 2021). Additionally, the beetles themselves generate oxygenated monoterpenes, as they metabolize toxic terpenes while feeding on the spruce tree's phloem (Blomquist et al., 2021). The detoxification process of terpenes involves a series of steps. In the first step, a hydroxyl group is introduced to a terpene molecule by cytochrome P450 catalysis. This modification increases the molecule's polarity and solubility in water, enabling beetles to eliminate it (Blomquist et al., 2021). In subsequent steps, the resulting terpenic alcohols are either excreted from the body or bound to detoxification conjugative molecules, such as fatty acid esters (Chiu et al., 2018) or glycosylates (Dai et al., 2021). This mechanism was studied in *Dendroctonus ponderosae* (Chiu, 2018; Chiu et al., 2019), *Dendroctonus armandi* (Dai et al., 2021), as well as in *Ips* species (Blomquist et al., 2021; Ramakrishnan et al., 2022) that feed on conifer trees, as these trees possess terpenes as a defense trait. This adaptation allows the beetles to overcome the tree's defenses and successfully colonize it. It is theorized that during evolution, some of these detoxification products, such as *cis*-verbenol in *I. typographus*, started to serve as aggregation pheromones for the bark beetles (Blomquist et al., 2021; Schebeck et al., 2023).

The exact behavioral role of all host-produced oxygenated monoterpene semiochemicals in bark beetles is not fully understood. However, according to the primary attraction theory proposed by Lehmanski et al. (2023), these compounds may play a role in helping male bark beetles detect weakened host trees, thereby facilitating successful colonization. Other compounds, e.g., 1,8-cineole and *trans*-4-thujanol, have proved to be potent anti-attractive compounds inhibiting beetle attraction to their pheromone (Jirošová et al., 2022b). 1,8-cineole has been identified as a potential predictor of bark beetle-resistant trees, along with several other specialized metabolites (Schiebe et al., 2012). Higher levels of *trans*-4-thujanol were detected in younger Norway spruces. Given that this compound has demonstrated repellency in high doses in laboratory olfactometer studies, it provides a potential explanation for the reduced attraction of *I. typographus* to trees below the age of 60 years (BlaŽyte-Cereškiene et al., 2016). The activity of 1,8-cineole and *trans*-4-thujanol for *I. typographus* has been evaluated in a field trapping experiment using different doses in combination with pheromones. Both compounds demonstrated a similar level of dose-dependent anti-attractant activity, with *trans*-4-thujanol inhibiting more the captures of females than males (Jirošová et al., 2022b). These compounds have been tested in combination with other anti-attractants for the protection of spruce trees in various forest environments, such as fresh forest
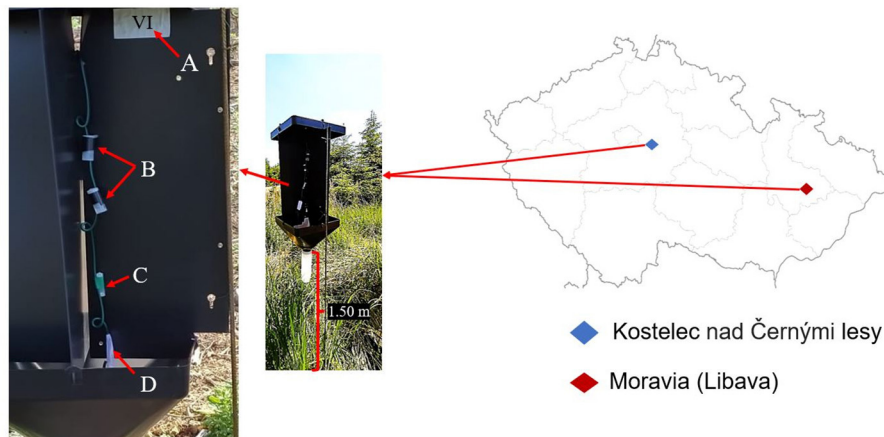
FIGURE 1
Field experiment sites and cross-panel field traps in the Czech Republic: **(A)** Trap label, **(B)** synthetic aggregation pheromone dispensers, **(C)** synthetic host tree odor dispenser, and **(D)** bait label for treatment identification.

edges or fragmented forests (Jakuš et al., 2022; Jirošová et al., 2022a). Furthermore, individual compounds *trans*-4-thujanol, (+)-isopinocamphone, camphor, and terpinen-4-ol were tested in different doses in two-choice Petri dish walking laboratory tests to assess their attractiveness to *I. typographus*. The activity of these compounds was largely insignificant, and only *trans*-4-thujanol and camphor at a high dose were attractive. In contrast, a more complex mixture of oxygenated monoterpenes, generated through the metabolization of (−)-β-pinene and (−)-bornyl acetate by the ophiostomatoid fungus *Grosmania polonica*, exhibited dose-dependent attractivity in the test (Kandasamy et al., 2023).

In multi-trophic interactions, the bark beetle predator-prey relationships are influenced by qualitative and quantitative aspects of host tree compounds and prey pheromones (Erbilgin and Raffa, 2001; Netherer et al., 2021). The natural enemies associated with *I. typographus* include Hymenoptera: Pteromalidae (*Rhopalicus* spp.), Diptera: Dolichopodidae (*Medetera* spp.), Coleoptera: Cleridae (*Thanasimus* spp.), and Coleoptera: Staphylinidae (*Nudobius* sp. and *Quedius* sp.; Wegensteiner et al., 2015). Laboratory bioassays demonstrated the strong attraction of female *Rhopalicus* spp. to infested logs, with antennal responses to oxygenated monoterpenes including pinocamphone, pinocarvone, and the phenylpropanoid estragole (Pettersson, 2001; Pettersson and Boland, 2003). Similarly, camphor, pinocamphone, and terpinen-4-ol derived from bark beetle-associated microorganisms in infested spruce logs exhibited strong attraction with *Medetera signaticornis* Loew (Sousa et al., 2023). Staphylinidae feeds on a wide range of prey, and some species are hypothesized to be associated with bark beetles (Pelto-Arvo, 2020). However, their specific association with host tree volatiles, such as oxygenated monoterpenes and *I. typographus* pheromone, remains unexplored.

The foraging strategies of some *I. typographus* predators, *Medetera* spp. and *Thanasimus* spp., could be governed by a more complete blend of the aggregation pheromone and host volatiles (represented by oxygenated monoterpenes; Hulcr et al., 2006). The hemiterpene pheromone component 2-methyl-3-buten-2-ol by itself does not increase predator catch, while the minor component

ipsdienol does (Bakke and Kvamme, 1981; Hulcr et al., 2006; Raffa, 2014).

Further research is necessary to achieve a more comprehensive understanding of the mechanisms and functions of oxygenated monoterpenes and estragole in the behavior of bark beetles and their predators.

In this study, we aimed to address the following specific questions:

1. Can individual host tree-oxygenated monoterpenes and estragole enhance or reduce the attractiveness of *I. typographus* pheromones in field traps for capturing bark beetles?
2. If so, which of the three tested decadic steps in release rates, namely, low (representing conditions closest to natural levels), medium, and high (representing doses used in management), exhibits the highest efficacy?
3. What is the species composition and abundance of predatory insects attracted to the tested compounds using the methodology described?
4. Can we propose a specific role for the tested compounds in bark beetle ecology?

We conducted a field trapping experiment to investigate the activity of eight synthetic host tree compounds (estragole, 1,8-cineole, (±)-camphor, (−)-carvone, (−)-α-terpineol, (−)-terpinen-4-ol, (+)-pinocamphone, and (+)-isopinocamphone) for *I. typographus* and some of its natural enemies. The compounds were tested at low, medium, and high release rates, in combination with components in pheromone barrier traps.

2 Materials and methods

2.1 Experimental area and design

For field experiments, we chose two locations in the Czech Republic. The first location was in North Moravia in Libavá

TABLE 1 The gravimetric establishment of released rates of tested compounds performed in the laboratory and field.

Compounds	Sources	Purity (%)	Doses	Release rates (mg/day)			Dispenser design
				Nominal	Lab. ± SEM $N = 3$	Field ± SEM $N = 3$	
Estragole	Sigma-Aldrich	98	L M H	0.1	0.13 ± 0.04	0.14 ± 017	Glass vial (2 ml), lid hole (1 mm)
				1	1.19 ± 0.17	1.72 ± 0.94	[†]Foil sachet: hole of 2 mm
				10	8.78 ± 1.71	1.86 ± 0.4	[†]Foil sachet: hole of 9 mm
1,8-Cineol	Sigma-Aldrich	98	L M H	0.1	0.06 ± 0.07	0.11 ± 0.01	[††]PE-vial (Kartell 731), without hole with paraffin oil (1 ml)
				1	0.66 ± 0.12	0.92 ± 0.12	Glass vial (2 ml), lid hole (1 mm)
				10	5.20 ± 0.30	5.70 ± 6.7	[††]Kartell 730 with hole (2 mm)
(–)-terpinen-4-ol	Sigma-Aldrich	98	L M H	0.1	0.07 ± 0.04	0.35 ± 0.06	Glass vial (2 ml), lid hole (1 mm)
				1	0.41 ± 0.21	0.52 ± 0.49	[††]Kartell 731 without hole
				10	9 ± 2.34	7.8 ± 10.16	[†]Foil sachet: hole of 18 mm
(–)-carvone	Sigma-Aldrich	95	L M H	0.1	0.13 ± 0.05	0.27 ± 0.13	Glass vial (2 ml), lid hole (1 mm)
				1	0.66 ± 0.28	0.22 ± 0.16	[††]Kartell 730 without hole
				10	9.1 ± 2	4.92 ± 3.45	[†]Foil sachet: hole of 18 mm
(±)-camphor	Alfa Aesar	95	L M H	0.1	0.09 ± 0.05	0.14 ± 0.9	Glass vial (2 ml), lid hole (1 mm)
				1	0.58 ± 0.06	1.71 ± 0.77	[††]Kartell 730: hole of 2 mm
				10	7.33 ± 0.94	0.67 ± 10	PE-sachet without hole

(Continued)

TABLE 1 (Continued)

Compounds	Sources	Purity (%)	Doses	Release rates (mg/day)			Dispenser design
				Nominal	Lab. ± SEM N = 3	Field ± SEM N = 3	
(-)-α-terpineol	Sigma-Aldrich	90	L M H	0.1	0.11 ± 0.05	0.05 ± 0.02	[††]Kartell 730 without hole
				1	1.37 ± 0.27	0.14 ± 0.07	[†]Foil sachet: hole of 9 mm
				10	4.01 ± 0.4	1.11 ± 0.48	PE-sachet without hole
(+)-isopinocamphone	[†††]	99	L M H	0.1	0.37 ± 0.40	0.40 ± 0.11	[†]Foil sachet: hole of 1 mm
				1	1.47 ± 0.08	1.87-0.67	[††]Kartell 730 without hole
				10	8.39 ± 0.98	8.21 ± 1.41	[††]Kartell 731, lid hole (2 mm)
(+)-pinocamphone	[†††]	[††††]	L M H	0.1	0.4 ± 0.11	0.3 ± 0.6	[†]Foil sachet: hole of 1 mm
				1	1.01 ± 0.34	1.32 ± 0.83	[††]Kartell 730 without hole
				10	9.1 ± 0.59	7.91-2.14	[††]Kartell 731, lid hole (2 mm)
2-methyl-3-buten-2-ol	Across	97	H	50	32.2 ± 20.5	9.10 ± 16.1	[††]Kartell 731, lid hole (1 mm)
(S)-cis-Verbenol	Sigma-Aldrich	95	H	1	1.53 ± 0.15	0.85 ± 1.34	[††]Kartell 731, lid hole (9 mm)

[†]Cellulose sponge square 7.5 × 3.5 × 0.25 cm sealed in PE foil thickness 0.1 mm, loaded with 200 µl of compounds, finally sealed in an outer layer made of aluminum/PE foil with the hole with a given diameter in the middle of one side of the dispenser.
[††]PE vials Kartell (Labware-Italy) size 731 and 730.
[†††]Compounds synthesized in Unelius laboratory (Ganji et al., 2020).
[††††]The (+)-pinocamphone contained 29% (+)-isopinocamphone.

(Military Forests, latitude 49°38′49 "N, longitude 017°33′50" E, 350 m above sea level). It consisted of a 40-year-old Norway spruce forest that has been heavily impacted by a bark beetle outbreak since 2015 (Brázdil et al., 2022). The experiment in Moravia was conducted from 18 May to 3 June 2022. The second location, Kostelec nad Cernými Lesy (Forests CZU; latitude: 49°55′57 "N, longitude: 014°55′13" E, 600 m above sea level), consisted of a 70–90-year-old Norway spruce forest. Traps were placed in a 2-year-old clearing measuring ∼200 m × 300 m. The experiment was carried out from 3 June to 28 July 2022. The experiment was designed identically in both locations (Figure 1).

The activity of estragole, 1,8-cineole, (±)-camphor, (–)-carvone, (-)-α-terpineol, (–)-terpinen-4-ol, (+)-pinocamphone, and (+)-isopinocamphone was tested at three different doses, represented by their release rates evaporated/sublimated from the dispenser (nominal 0.1, 1, and 10 mg/day, Table 1). The doses were determined based on the published releases of oxygenated monoterpenes from healthy trees at 24°C, which varied from 0.1 to 7 µg/m²/h of stem surface (Ghimire et al., 2016). When considering a tree stem with a 50 cm diameter and an exposed surface area of ∼24 m² (representing 15 m of stem height vulnerable to bark beetle attack), the estimated daily release rate of these oxygenated monoterpenes over a 24-h period would be ∼0.5–4 mg/day. We used the pure enantiomers of (–)-carvone, (–)-terpinen-4-ol, (+)-pinocamphone, and (+)-isopinocamphone, which triggered a higher response on the *I. typographus* antennae (Schiebe et al., 2012; Hou et al., 2021; Kandasamy et al., 2023) and were commercially available or synthesized in the laboratory (Ganji et al., 2020). Experimental dispensers were designed in the laboratory, and their exact laboratory and field release rates were established using the gravimetric method and measured six times (Jirošová et al., 2022b) in a laboratory fume hood (temperature 25°C and airflow 0.5 m/s) and in the field under the same weather conditions as the experiments (Table 1).

In the field, the intercept pheromone traps (Ecotrap/Fytofarm, Ltd., Bratislava; Slovakia) were mounted on poles 1.5 m above the ground in rows >30 m from any forest edge. The distance between traps was >15 m (Supplementary Figure 1). In each field location, 32 intercept pheromone traps were baited with dispensers with *I. typographus* pheromone (2-methyl-3-buten-2-ol at 9.1 mg/day and (S)-cis-verbenol at 0.9 mg/day). In 24 of these traps, an additional dispenser was placed with one of the eight test compounds in one of three doses.

For each compound, one block represented four traps arranged in a row: three traps with different doses in combination with pheromone and one trap with pheromone-only (Control). The position of the tested baits among these four traps was changed four times according to the randomization scheme in a Latin square design (Evans et al., 2020). These four rotations were repeated twice for each compound, resulting in a total of eight collections of catches for each treatment. Insects collected during the field experiment in both localities were preserved in ethanol for further analysis. Predators and parasitoids were sorted by family and identified at the genus level. The identification of Pteromalidae wasps followed the methods described by Peck et al. (1964), Graham (1969), and Bouček and Rasplus (1991). For Staphylinidae (rove beetles), the identification followed the guidelines provided by Arnett and Thomas (2000) and Navarrete and Newton (2003).

TABLE 2 Testing of relative catches of *I. typographus* in the tested compounds in different doses.

Compound	Model family	AIC	Pr(>Chi)	t-test contrast comparison to pheromone-only		
				Low	Medium	High
Estragole	genpois	521.63	0.003476 **	0.4973	0.2991	0.0206 *
1,8-Cineol	genpois	557.89	8.03E-08 ***	0.0003 ***	0.0051 **	3.58E-09 ***
(–)-terpinen-4-ol	nbinom2	532.27	0.2849	0.106	0.667	0.81
(–)-carvone	nbinom2	509.67	0.8852	0.869	0.64	0.782
(±)-camphor	nbinom2	537.5	0.5298	0.19	0.345	0.221
(–)-α-terpineol	nbinom2	526.26	0.8826	0.655 .	0.903	0.721
(+)-isopinocamphone	genpois	550.17	0.003014 **	0.0785	0.00079 ***	0.003142 **
(+)-pinocamphone	genpois	521.03	3.32E-07 ***	0.924	1.3E-06 ***	0.483

Asterisks mark a significant difference (*p < 0.05; **p < 0.01; ***p < 0.001) of the dose from pheromone alone.

2.2 Statistical analysis

For the evaluation of the effects of each individual compound, a separate regression model was fitted with the relative number of *I. typographus* as the dependent variable. The relative number was expressed as the number of insects of a single taxon captured by a treatment within a block divided by the total catches by the block for a single catch collection. Due to the experimental design, we utilized a mixed-effects model approach (Zuur et al., 2009). The random part of the model was, in all cases, trap-nested in the locality. During model building and validation, an appropriate distribution function was selected by minimizing the Akaike information criterion (AIC), and the significance of the model was tested by the likelihood ratio test (χ^2). Between the best models, only two distribution functions were selected (Table 2): generalized poison distribution (Joe and Zhu, 2005) and negative binomial distribution in quadratic parameterization, according to Hardin and Hilbe (2007). We used a *t*-test to compare the response to each compound dose against the pheromone-only control. The model formulation was performed in R version 4.3.1 (R Core Team, 2023) in the package *glmmTMB* following the procedures described by Brooks et al. (2017).

3 Results

3.1 *Ips typographus* response to tested compounds in combination with aggregation pheromone

During field experiments, a total of 39,650 *I. typographus* adults were caught. The number of adults captured in Kostelec ($N = 28,931$) was 2.7 times higher than in Moravia ($N = 10,719$). However, the pattern of catches for the tested compounds was almost the same. Compounds with significantly different catches in the treatments: estragole (χ^2; $p < 0.01$), 1,8-cineole (χ^2; $p < 0.001$), (+)-pinocamphone (χ^2; $p < 0.01$), and (+)-isopinocamphone (χ^2; $p < 0.01$; Table 1, Figure 2). For 1,8-cineol, all three doses in combination with pheromones resulted in significantly fewer beetles caught than the pheromone-only control, with stronger effects observed for the high dose (*t*-test; $p < 0.001$, $p < 0.01$, $p < 0.001$; Table 2, Figure 2). For estragole, the inhibitory effect was significant only for the high dose (*t*-test; $p < 0.05$), while for pinocamphone, it was observed at the medium dose (*t*-test; $p < 0.001$). However, regarding the medium dose of pinocamphone, there were different catch rates in Libava and Kostelec (Figure 2), suggesting a potential problem with the dispenser used in Kostelec. Conversely, isopinocamphone resulted in statistically higher catch rates at both high and medium doses (*t*-test; $p < 0.01$ and $p < 0.001$, respectively) compared to the pheromone-only treatment (Figure 2). A low dose of isopinocamphone showed the same nearly significant trend (*t*-test; $p = 0.08$; Table 2). Catches of beetles of remaining tested compounds in combination with pheromone, including ($\pm$)-camphor, (−)-carvone, (-)-α-terpineol, (-)-terpinen-4-ol, and pheromone-only, did not exhibit significant differences (Table 2). Additionally, there were no significant differences in catches between the individual doses and the pheromone-only control group for any of these compounds (Supplementary Figure 2).

For each compound, a separate model (GLMM) was created with the formula: *Relative count of I. typographus ~ Compound dose + (1|Locality)*. The appropriate distribution function (model family: genpois—Generalized poison; nbinom2—Negative binomial), Akaike information criterion (AIC), and significance test (χ^2) are stated for each compound. The results of the t-test contrast comparison given for each compound and dose combination against pheromone-alone (control). Asterisks mark a significant difference ($p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***) of the dose from pheromone alone.

3.2 Predatory insect response to tested compounds in combination with aggregation pheromone

The catch of natural enemies was three times higher in Kostelec compared to Moravia, which corresponds to a higher number of bark beetle catches in Kostelec. Four families of natural enemies of bark beetles were identified with a prevalence of Pteromalidae and Staphylinidae (Supplementary Tables 1, 2). In Kostelec, there were 93 specimens of parasitoid wasps belonging to the genus *Rhopalicus* sp. (Hymenoptera: Pteromalidae), while in Moravia, there were only 13 specimens. Additionally, in Kostelec, 19 specimens of rove beetles (Coleoptera: Staphylinidae) were caught, and in Moravia, 23 specimens (Table 3).
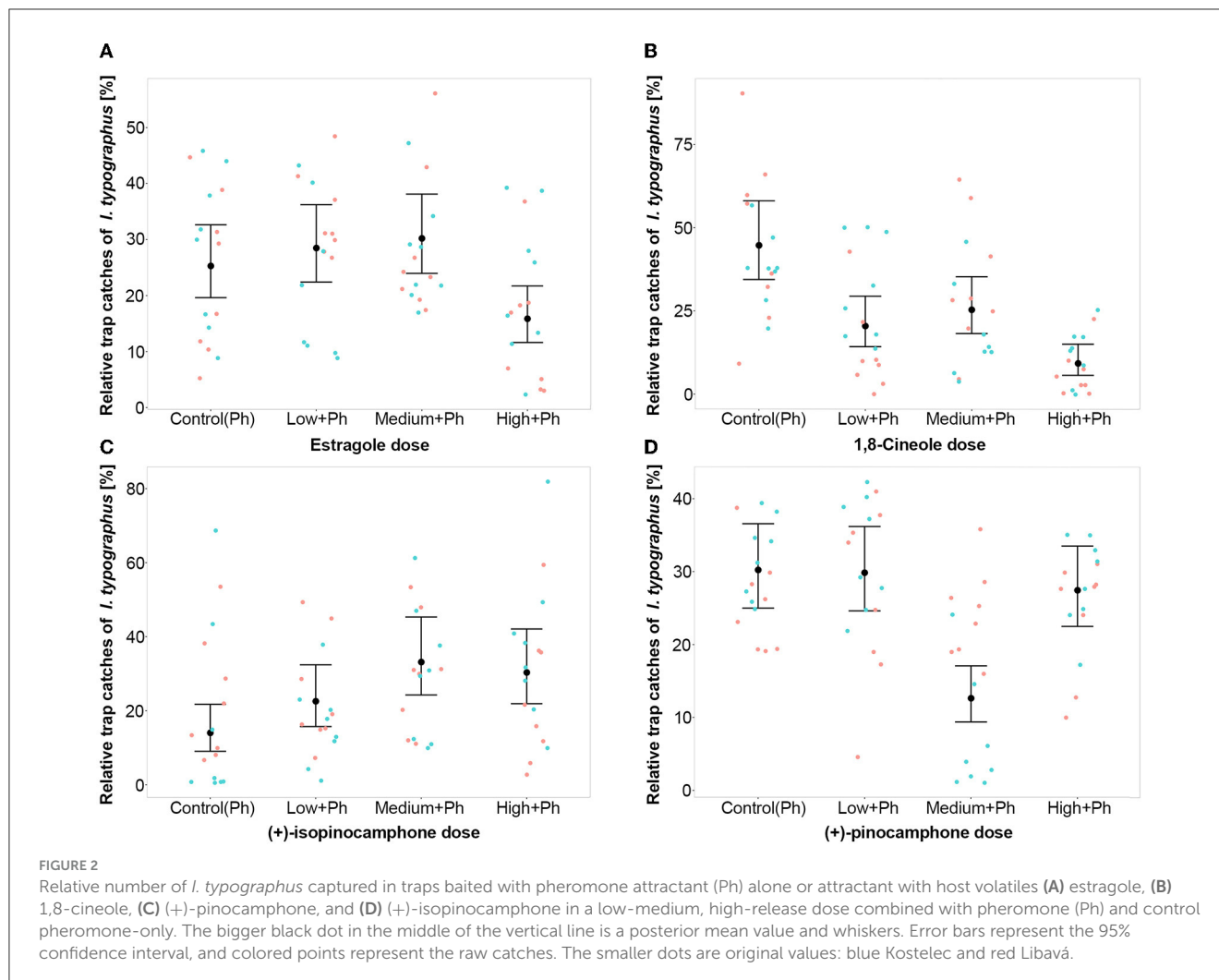
The ratio between these two groups appeared different between the two locations, with Kostelec having a higher proportion of Pteromalid wasps compared to Staphylinids, while in Moravia, we observed the opposite trend. There were a few catches recorded for *Medetera* sp. (Diptera: Dolichopodidae), with five specimens in Kostelec and three specimens in Moravia. There were four catches of *Lonchaea* sp. (Diptera: Lonchaeidae), three in Kostelec, and one in Moravia. For *Thanasimus* sp. (Coleoptera: Cleridae), there were two catches in Kostelec and three catches in Moravia.

The catches of *Rhopalicus* sp. wasps did not show significant differences between compounds and their doses. The number of caught specimens in Moravia and Kostelec is listed in the Supplementary Tables 1, 2.

4 Discussion

4.1 Response of *Ips typographus* to tested compounds

The variability in total catches of *I. typographus* and its predators, as well as their varying distributions across different treatments in the experimental locations of Kostelec and Moravia, could be attributed to the unique weather conditions experienced during each field experiment, as described in Supplementary Figure 3. Furthermore, this variation may have been influenced by the season impacting the flight activity of the beetles. The variation between catches in blocks of traps may have been due to different locations of blocks within the clearing, in terms of their distance to the forest edge and wind speed and

FIGURE 2
Relative number of *I. typographus* captured in traps baited with pheromone attractant (Ph) alone or attractant with host volatiles **(A)** estragole, **(B)** 1,8-cineole, **(C)** (+)-pinocamphone, and **(D)** (+)-isopinocamphone in a low-medium, high-release dose combined with pheromone (Ph) and control pheromone-only. The bigger black dot in the middle of the vertical line is a posterior mean value and whiskers. Error bars represent the 95% confidence interval, and colored points represent the raw catches. The smaller dots are original values: blue Kostelec and red Libavá.

direction. The trap catches could also have been influenced by different ages of spruce stands near the experiments.

In many cases, the beetles exhibited weak differences in response to the tested compounds in combination with the pheromone compared to the pheromone alone, and the observed effects were only marginally significant. However, there was a noticeable anti-attractive effect observed with 1,8-cineole, a compound for which similar findings and similar trends have been reported previously (Andersson et al., 2010; Binyameen et al., 2014; Jirošová et al., 2022b).

New findings were the anti-attractive effect of a high dose of estragole and the effect of (+)-isopinocamphone on enhancing the attractive activity of the pheromone for *I. typographus*. There was also a less clear inhibition effect of a medium dose of pinocamphone, probably caused by a defective test dispenser for a medium dose at Kostelec. In contrast, when α-terpineol, camphor, carvone, or terpinen-4-ol were added to the pheromone, they did not alter its attractiveness, despite these compounds eliciting strong responses from *I. typographus* antennae (Kalinová et al., 2014; Schiebe et al., 2019).

The ecological role of estragole, 1,8-cineole, and isopinocamphone in the interaction between *I. typographus* and Norway spruce trees was investigated by Schiebe (2012, 2019). The amount of these compounds, along with other oxygenated monoterpenes found in spruce, increased in felled trees and in standing trees after the application of the plant hormone analogue methyl jasmonate. The quantity of these compounds was negatively correlated with the density of bark beetle attacks when the beetles infested the felled trees, and the standing trees that exhibited a higher induction of these compounds were able to survive a natural bark beetle infestation.

The effect of estragole has been tested on several bark beetle species (Coleoptera: Curculionidae: Scolytinae), including *Dendroctonus brevicomis* LeConte (Hayes and Strom, 1994), *Ips pini* (Say), *Ips latidens* (Leconte) (Joseph et al., 2001), and *Tomicus piniperda* (Curculionidae: Scolytinae) (Haack et al., 2004). Its inclusion in their host odor blend resulted in reduced trap catches. Estragole was also reported to interrupt the responses of bark beetle species *Dedroctonus simplex* (Le Conte) and *D. ruffipenis* (Kirby) to their attractive pheromone components (Werner, 1995).

TABLE 3 Natural enemies of bark beetles (Staphylinidae and Pteromalidae) captured in synthetic host tree compounds: estragole, 1,8 cineole, camphor, carvone, alpha-terpineol, terpinene-4-ol, (+)-pinocamphone, and (+)-isopinocamphone, in combination with pheromone and pheromone-only controls.

Doses/location	Estragole+Ph	1,8-cineole+Ph	a-terpineol+Ph	Camphor+Ph	Carvone+Ph	terpinen-4-ol+Ph	(+)-isopinocamphone+Ph	(+)-pinocamphone+Ph	Pheromone-only Ph
Doses/Moravia **Pteromalidae**	*Rhopalicus*	*Rhopalicus* (N = 4)	*Rhopalicus* (N = 2)	*Rhopalicus*	0	*Rhopalicus*	*Rhopalicus* (N = 3)	0	*Rhopalicus*
Doses/Moravia **Staphylinidae**	*Gyrohypnus* (N = 2)	*Nudobius* (N = 4)	*Gambrinus*	*Bisnius Nudobius Anotylus*	*Bisnius* (N = 2) *Anotylus*	*Nudobius Gabrius*	*Nudobius*	*Bisnius; Gyrohypnus*	*Nudobius* (N = 2); *Gyrohypnus Anotylus Rugilus*
Doses/Kostelec **Pteromalidae**	*Rhopalicus* (N = 16)	*Rhopalicus* (N = 9)	*Rhopalicus* (N = 8)	*Rhopalicus* (N = 2)	*Rhopalicus* (N = 10)	*Rhopalicus* (N = 5)	*Rhopalicus* (N = 14)	*Rhopalicus* (N = 7)	*Rhopalicus* (N = 22)
Doses/Kostelec **Staphylinidae**	*Nudobius Anotylus Gyrohypnus*	*Nudobius* (N = 2); *Stenus Gyrohypnus Heterothops*	*Rugilus Quedius*	*Aleochara Nudobius Gyrohypnus*	*Bisnius Nudobius*	*Aleochara*	*Nudobius*	*Nudobius*	*Anotylus*

Doses: The sum of total captures in all three tested release rates of compounds or pheromone-only controls summed together for both localities, Kostelec and Moravia.

However, recent research has revealed that the addition of estragole increased catches of both *Dendroctonus frontalis* (Zimmermann) and *D. terebrans* (Olivier) on their pheromone lures (Munro et al., 2020). Based on these findings, we suggest that this semiochemical has variable ecological roles for these different species.

In our study, (+)-isopinocamphone caused a synergistic increase in beetle catches when added to the pheromone. This observation, combined with the fact that bark beetles possess specialized sensilla on their antennae (Hou et al., 2021) to detect it, suggests the potential role of (+)-isopinocamphone in the selection of host trees. Kandasamy et al. (2023) tested in a short-range two-choice test in a Petri dish synthetically prepared (+)-isopinocamphone added as a solution in mineral oil to spruce bark agar, which did not exhibit significant attractivity for *I. typographus* bark beetles in tested doses. This further indicates that the effect of (+)-isopinocamphone we see in trap catches may be a long-range attraction (in accordance with the known long-range attraction of *cis*-verbenol; Schlyter and Birgersson, 1999).

4.2 Response of bark beetle insect natural enemies to tested compounds

The anticipated captures of the common predatory beetle *Thanasimus* sp. were relatively low. This could be attributed to the fact that our pheromone bait only contained the two major pheromone components, 2-methyl-3-buten-2-ol and (*S*)-*cis*-verbenol, and not ipsdienol, an *I. typographus* pheromone component emitted in smaller amounts in the later attack states (Birgersson et al., 1984; Hulcr et al., 2006). Furthermore, we observed only a few captures of *Medetera* sp. and *Lonchaea* sp. flies, likely due to the use of a trap optimized for Coleoptera that lacked sticky surfaces.

Although there is limited information on Staphylinidae predators using host tree volatiles for locating bark beetles (Wegensteiner et al., 2015), it has been reported that they are attracted to pheromone traps used for monitoring *Ips typographus* (El-Sayed, 2023). Additionally, commercial pheromone traps tested in combination with host tree logs (*P. abies*) caught ~38% more predatory Staphylinidae than traps without logs, in comparison to a 32% increase in catches of *Thanasimus formicarius* (L.) (Zumr, 1983). Hence, host tree compounds may mediate staphylinid prey location.

The pteromalid parasitoid wasp *Rhopalicus* sp. was the most abundant among the captured bark beetle natural enemies, but there were no significant preferences for any of the tested compounds due to the low number of caught specimens. In the literature, an electroantennographic study of bark beetle gallery smell was tested on the antennae of *Rhopalicus tutela* (Walker) females. The antennae showed sensitivity to oxygenated monoterpenes and estragole (Pettersson, 2001). Additionally, the olfactory response to estragole was reported in other species of parasitoid wasps, *Spathius pallidus* Ashmead, 1893,

and *Roptrocerus xylophagorum* (Hymenoptera: Pteromalidae; Sullivan et al., 1997).

5 Conclusion

The effect of 1,8-cineole, estragole, and (+)-isopinocamphone, as observed in our field experiments, provides evidence that these oxygenated monoterpenes and estragole can exhibit biological activity for *I. typographus* and their natural enemies when combined with *I. typographus* aggregation pheromone. This suggests that their long-range activity is not solely dependent on a complex mixture, such as that emitted by symbiotic fungi inoculated on wood substrates (Kandasamy et al., 2023).

The discovery of new attraction inhibitors or adjuvants for attractants can be applied to the development of integrated pest management methods for controlling *I. typographus*. Anti-attractants, a term broadly used for attraction inhibitors, have already been tested to deter various pest bark beetles, such as *Dendroctonus ponderosae*, *D. rufipennis*, *D. pseudotsugae*, *Ips pini*, and *Dryocoetes confusus*, from attraction to their pheromone or to the host tree (Schlyter, 2012). These anti-attractants can originate from host trees, non-host trees, associated microorganisms, or the beetles themselves (Borden et al., 2000; Munro et al., 2020).

In the protection of Norway spruce trees against *I. typographus*, verbenone, a well-established repellent for bark beetles, was tested with varying success (Jakuš et al., 2003; Frühbrodt et al., 2023). In nature, verbenone signals an old and over-exploited host. The synergistic blend effect of verbenone mixed with green leaf volatiles (C6 alcohols) and C8 alcohols (3-octanol and 1-octen-3-ol) and the angiosperm and fungal spiroacetal conophthorin (Zhang and Schlyter, 2004) was also evaluated for tree protection against *I. typographus* (Schiebe et al., 2011), resulting in a reduction of tree killing ranging from 35 to 76% in protected areas.

The recently tested anti-attractant mixture also includes, besides the 3-octanol, 1-octen-3-ol, hexanol and conophthorin, 1,8-cineole and *trans*-4-thujanol from spruce and excludes verbenone (Jirošová et al., 2022a). Anti-attractant blends offer partial protection for standing trees but are ineffective for windfallen trees. Adding new anti-attractants, e.g., geranyl acetone (Lindmark et al., 2023), to the mixture may enhance tree protection effects.

A comprehensive approach to semiochemical tree protection against *I. typographus* attacks could employ the push-pull strategy. Trees are protected by anti-attractants, and repelled beetles are caught in pheromone traps baited with attractive *I. typographus* pheromones. Both the push and pull might be enhanced by the addition of new semiochemicals (Jakuš et al., 2022; Deganutti et al., 2023). The addition of (+)-isopinocamphone to the trapping lure could increase beetle attraction away from trees while simultaneously protecting them from an estragole-enhanced beetle repellant.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because Ethical review and approval were not required for the study on animals in accordance with the local legislation and institutional requirements. We have performed all beetle experiments that comply with the ARRIVE guidelines and were carried out in accordance with (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Author contributions

AACM: Data curation, Formal analysis, Investigation, Validation, Writing—original draft. RJ: Data curation, Investigation, Writing—review & editing. RM: Formal analysis, Writing—review & editing. CRU: Methodology, Writing—review & editing. FS: Conceptualization, Methodology, Supervision, Validation, Writing—review & editing. AJ: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2023.1292581/full#supplementary-material

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