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Behavioural activity of *Ips typographus* (L), (Coleoptera: Scolytinae) semiochemicals, selection of new candidates as a green alternative of bark beetle management

Dissertation thesis

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Ph.D. Thesis Assignment

Certificate of Authorship

I hereby confirm that this Ph.D. thesis “Behavioural activity of *Ips typographus* (L), (Coleoptera: Scolytinae) semiochemicals, selection of new candidates as a green alternative of bark beetle management “was developed independently and ethically. I state that all the information sources and literature used are specified correspondingly, and the thesis was formulated under the direct supervision of my scientific supervisors.

I agree with the disclosure of this Ph.D. Thesis according to Czech Law (Act No. 111/1998 Coll. Sb.) regardless of the Defense of thesis results.

August 2026, Prague

Antonioni Acacio Campos Moliterno

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Abstract

The European spruce bark beetle, *Ips typographus*, is one of the most destructive forest pests of Norway spruce, *Picea abies*, in Europe. Its increasing impact under climate change highlights the need for improved methods for early infestation detection and environmentally sustainable management. This dissertation investigated the chemical ecology of the *P. abies* - *I. typographus* interaction by examining three connected processes: the spatial distribution of host volatiles around recently infested trees, the effects of selected host-associated compounds on pheromone-mediated attraction, and the influence of female phenotype on behavioural and antennal responses. The first study evaluated the three-dimensional distribution of *α*-pinene emitted by recently infested Norway spruce. Volatile organic compounds were collected under laboratory and field conditions using solid-phase microextraction fibers and HayeSep-Q® sorbent cartridges. Field sampling was conducted at different distances, heights, and cardinal directions around infested trees. *α*-pinene was detectable around recently attacked trees, but its abundance varied spatially and between field trials. Abundance was generally recorded close to the stem, although the distribution was strongly influenced by sampling position and environmental conditions. These findings demonstrate the potential of host volatiles for early infestation detection while emphasizing the dynamic nature of volatile plumes in forest environments. The second study examined the effects of eight synthetic host-associated compounds combined with the aggregation pheromone of *I. typographus*. Field trapping experiments showed that 1,8-cineole reduced beetle attraction at all tested release rates, while estragole produced an anti-attractant effect at the highest release rate. In contrast, (+)-isopinocamphone enhanced pheromone-mediated attraction. The remaining tested compounds did not significantly alter trap catches under the experimental conditions. Natural enemies, including pteromalid parasitoids and staphylinid beetles, were also captured, although their responses varied between locations and treatments. The third study investigated whether female body size and antennal morphology were associated with differences in behavioural and electrophysiological responses. Field traps containing high release rates of (+)-isopinocamphone captured larger females, whereas 1,8-cineole was associated with smaller females under some conditions. Large females had larger

antennal clubs and showed stronger electroantennographic responses to high doses of (+)-isopinocamphone. In contrast, small females responded more strongly to several doses of 1,8-cineole. Responses to the aggregation pheromone did not differ significantly between female size classes. Overall, this dissertation demonstrates that host selection by *I. typographus* is shaped by interactions among volatile production and dispersal, compound identity and concentration, pheromone communication, environmental conditions, and beetle phenotype. The findings support the use of *α*-pinene and related volatiles in early-detection systems, 1,8-cineole and estragole as potential anti-attractants, and (+)-isopinocamphone a possible pheromone synergist. These results provide a foundation for more selective, semiochemical-based approaches to bark beetle monitoring and management and may help reduce reliance on conventional insecticides in European spruce forests.

Keywords: antagonist*, α*-pinene*;* bark beetle, electroantennography; field test; forest; infestation, protection, synergistic, host selection.

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II. Moliterno AAC, Jakuš R, Modlinger R, Unelius CR, Schlyter F, Jirošová A. Field effects of oxygenated monoterpenes and estragole combined with pheromone on attraction of *Ips typographus* and its natural enemies. Frontiers in forests and global change. 2023; 1292581; doi: 10.3389/ffgc.2023.1292581

III. Moliterno AAC, Shewale MK, Basile S, Synek J, Jirošová A. Size-dependent behavioural and antennal responses to doses of (+)-isopinocamphone and 1, 8-cineole mixed with pheromone: a potential host selection strategy in female *Ips typographus* L. Annals of Forest Science. 2025; 82(1), p.34;doi: 10.1186/s13595-025-01297-4

List of publications not included in this thesis

I. Jakuš R, Trubin A, Singh VV, Zabihi K, Jirošová A, Modlinger R, Majdák A, Korolyova N, MoliternoAAC, Kašpar J, Slavík M. Spruce protection against *Ips typographus* with anti-attractant blend of tree-based semiochemicals: from small experimental plots to stand scales. Forests. 2024; 15(2), p.356; 2024; doi:10.3390/f15020356

II. Johny J, Diallo S, Nadachowska-Brzyska K, Moliterno AAC, Roy A, Kalinová B, Große-Wilde E, Schlyter F. Not all bark beetles smell the same: population-level functional olfactory polymorphisms in *Ips typographus* pheromone receptor ItypOR33. Molecular Ecology, 34(6), p.e17693; 2025; doi: 10.1111/mec.17693

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List of abbreviations and symbols

µg: micrograms

µm: micrometer

℃: degree Celsius

BB: Bark beetles

BVOCs: Biogenic Volatile Organic Compounds

cV: *cis*-Verbenol

DEFID2: Database of European Forest Insect and Disease Disturbances

EAG: electroantennography

e.g.: exempli gratia (example)

F: Fabricius (Species described by Johan Christian Fabricius)

FAO: Food and Agriculture Organization

FE: Forest edge

FRA: Forest Resources Assessment

GC-FID: Gas Chromatography coupled with Flame Ionization Detection

Hz: Hertz

IF: Inner Forest

IT: *Ips typographus*

IUPAC: International Union of Pure and Applied Chemistry

L: Linnaeus (Species described by Carl Linnaeus)

MT: Methyl butanol (2-methyl-3- buten-2-ol)

MT-Ox: Oxygenated monoterpenes

mg: milligrams mL: milliliters

mm: millimeters

mV: millivolts

Ph: Pheromone (synthetic)

OSN: olfactory sensory neuron

SEM: standard error mean

VOCs: Volatile Organic Compounds

1. Introduction

The Eurasian spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Curculionidae: Scolytinae), is one of the most important biotic disturbance agents in Norway spruce, *Picea abies* (L.), forests. Its population dynamics are strongly influenced by climate change and associated disturbance regimes (Hlásny et al., 2021). Drought weakens tree physiology and resin-based defenses, thereby reducing host resistance (Netherer et al., 2019; Netherer and Hammerbacher 2022), while storms increase beetle risk by producing windthrown or weakened spruce material suitable for reproduction (Wermelinger 2004; Netherer et al., 2024). In addition, warmer conditions can accelerate beetle development and increase voltinism, i.e., the number of generations completed per year (Kausrud et al., 2012; Økland et al., 2015).

The ecological and economic importance of *I. typographus* lies in its ability to shift from low-density endemic populations to high-density outbreaks capable of killing host trees (Wermelinger 2004; Kausrud et al., 2012). An outbreak can be defined as a rapid increase in beetle abundance that enables mass attacks and allows beetles to overcome host defenses (De Groot et al., 2019). In the Czech Republic, bark beetle-related wood losses during the peak outbreak period from 2017 to 2023 averaged approximately 9 million m^3 per year, illustrating the economic scale of such events (Lubojácký et al., 2022). In Central Europe, adult flight activity and trap catches typically begin in spring and continue into late summer, although the timing and number of flight peaks vary with elevation, temperature, and local weather conditions (Wermelinger 2004; Holuša et al., 2012; De Groot et al., 2023; Netherer 2023; Potterf et al., 2025). Across the broad European distribution of *P. abies*, beetle populations are exposed to gradients in temperature, photoperiod, solar radiation, and moisture availability, all of which influence development, dispersal, host susceptibility, and population growth (Annila 1969; Anderbrant 1986; Faccoli 2009; Fleischer et al., 2016; Stříbrská et al., 2022).

Successful colonization by *I. typographus* depends on the integration of visual and chemical cues. Males act as the pioneer sex during host location (Lehmanski et al.,

2023), and once suitable trees are found, male-produced aggregation pheromones promote the arrival of both males and females. This coordinated response facilitates mass attack, reproduction, and, when host defenses are overcome, the decline and eventual death of the host tree (Wermelinger 2004; Frühbrodt et al., 2023). Host defense therefore represents a critical initial barrier to colonization. These defenses include constitutive mechanical and chemical traits, such as bark thickness, resin, terpenes, terpenoids, and phenolic compounds (Franceschi et al., 2005; Netherer et al., 2021).

Beyond climate and host condition, beetle performance is shaped by biotic interactions, including intraspecific competition, inbreeding risk, interspecific competition, natural enemies, and microbial symbionts (Anderbrant et al., 1985; Franceschi et al., 2005; Wegensteiner et al., 2015; Netherer et al., 2021; Kandasamy et al., 2023; Dacquin et al., 2024). Population density is particularly important because it affects beetle phenotype, behavior, and fitness. High larval densities can intensify competition and reduce body size, whereas lower densities may allow larger body size and improved individual performance (Sallé et al., 2005; Sallé and Raffa 2007). Body size may also influence chemical communication in bark beetles (Pureswaran and Borden 2003), and larger females can have higher fecundity and longevity (Foelker and Hofstetter 2014). During swarm events, competition may intensify when the most suitable host trees are already colonized, thereby affecting host selection and beetle performance (Anderbrant et al., 1985; Grodzki 2004; Netherer and Hammerbacher 2022).

Dispersal ability and natural enemies further mediate the ecological success of *I. typographus*. Flight is energetically costly, and prolonged dispersal can deplete fat reserves while increasing exposure to predators and parasitoids (Botterweg 1982; Chen et al., 2011; Wermelinger et al., 2012). Beyond macrobiotic pressures, bark beetles are also shaped by microbiotic agents, including holobiont-associated microbes, symbiotic fungi, gut microbiota, and entomopathogenic microorganisms (Vega 2012; Chakraborty et al., 2023; Six et al., 2026). In *I. typographus*, these interactions may be beneficial, with gut bacteria contributing to cellulose degradation,

detoxification, nutrient acquisition, and possible protection against pathogens (Davis et al., 2019; Chakraborty et al., 2020a), but also antagonistic including effect of entomopathogenic fungi (Sun et al., 2025). Because these microbial communities vary with life stage, season, host condition, and geography, the bark beetle holobiont should be considered dynamic rather than fixed (Veselská et al., 2023; Chakraborty et al., 2023). Together, abiotic and biotic factors influence bark beetle fitness, defined as individuals' reproductive contribution to future generations (Biedermann et al., 2019).

Chemical communication underlies many of the interactions described above and is therefore central to the ecology of *I. typographus*. The chemical compounds that convey information between organisms are known as semiochemicals (broadly biogenic volatile organic compounds (BVOC), and they influence behavior in various ecological contexts. Functionally, semiochemicals include pheromones, which mediate communication within a species, and allelochemicals, which mediate interactions between species (Byers 2007). Allelochemicals are further classified as allomones, which benefit the emitter; kairomones, which benefit the receiver; and synomones, which benefit both the emitter and the receiver (Bergström 2007).

In *I. typographus*, semiochemical cues play a key role in host selection, aggregation, mate location, and colonization (Lieutier et al., 2007). Host-tree volatiles can provide information about host identity, physiological condition, and infestation status (Jaakkola et al., 2023; Frühbrodt et al., 2023). These signals are not used by bark beetles alone but can function as synomones by attracting predators and parasitoids to herbivore-attacked trees (Hulcr et al., 2006; Byers 2007). A similar cofunction may also have bark beetle pheromones serving as kairomones for natural enemies. Thus, in spruce forests, BVOC blends create complex odor landscapes that shape interactions among bark beetles, host trees, non-host trees, microorganisms, predators, and parasitoids (Zhang and Schlyter 2003; Schiebe et al., 2011; Lehmanski et al., 2023).

To selectively detect these chemical signals, bark beetles, similar to other insects such as moths, mosquitoes, and flies, possess a highly specialized olfactory system located

primarily on their antennae (Hansson and Stensmyr 2011; Missbach et al., 2014). Chemical cues emitted by conspecifics, host trees, or other environmental sources are recognized through interactions with specific olfactory proteins and receptors. These chemical signals are then transformed into neural signals and processed by the nervous system, leading to behavioural responses such as orientation, host selection, aggregation, mating, or avoidance (Jacquin-Joly and Merlin 2004).

For *I. typographus*, successful host colonization depends on the interaction between beetle-produced aggregation pheromones and host-derived volatiles. Together, these signals promote conspecific recruitment to suitable host trees (Lehmanski et al., 2023). When beetle density becomes sufficiently high, coordinated mass attack can overwhelm tree defenses and allow successful colonization (Wermelinger 2004). Stressed or infested *P. abies* trees release volatile blends containing monoterpenes and oxygenated monoterpenoids (Jaakkola et al., 2022; Netherer et al., 2022), which may influence beetle attraction, repellence, or host acceptance (Netherer et al., 2021; Jirošová et al., 2022; Moliterno et al., 2023). For instance, 1,8-cineole is a minor oxygenated monoterpene produced by *P. abies*, yet it appears to play an important role in *I. typographus* host selection (Schiebe et al., 2019). This compound has been reported to reduce attraction to pheromone-baited traps (Jirošová et al., 2022; Moliterno et al., 2023). By contrast, (+)-isopinocamphone has been proposed as a host-associated volatile that may enhance beetle attraction when combined with aggregation pheromones (Moliterno et al., 2023).

In this sense, semiochemicals represent an important practical value for forest pest management as part of integrated pest management (IPM). IPM is a decision-based approach that combines prevention, monitoring, and control while minimizing environmental risks (FAO 2020). In the semiochemical context, including intra- and interspecific BVOCs, can support IPM by improving monitoring, early detection, and behavioural manipulation of bark beetle populations (Stříbrská et al., 2023; Frühbrodt et al., 2024a, 2024b; Ramakrishnan et al., 2025).

Host- and microbe-derived interactions can influence *I. typographus* behavior, affecting attraction, repellence, and host acceptance (Rudinsky et al., 1971; Schlyter et al., 1986, 1987; Erbilgin et al., 2007; Kalinová et al., 2014; Jirošová et al., 2022; Frühbrodt et al., 2023; Kandasamy et al., 2023). Similar chemical cues may also affect natural enemies of *I. typographus* and other bark beetle species, with potential implications for conservation biological control (Sullivan et al., 1997; Raffa 2014; Moliterno et al., 2023; Sousa et al., 2023, 2024).

A better understanding of these dose-dependent responses is essential for refining semiochemical-based IPM strategies. One example is push-pull management, which combines repellents that “push” beetles away from vulnerable trees with attractants that “pull” them into traps or trap trees (Cook et al., 2007; Jakuš et al., 2022, 2024). Thus, semiochemicals are important not only for understanding bark beetle ecology, but also for developing sustainable forest protection tools.

2. Aims and objectives of the thesis

The main aim of this PhD thesis is to identify and characterize novel bioactive semiochemicals associated with the ecological niche of *Ips typographus* and to evaluate their potential for developing environmentally sound tools for bark beetle monitoring, early detection, and population management. The thesis focuses on candidate semiochemicals originating mainly from two sources: metabolites produced by bark beetle-associated ophiostomatoid fungi and volatile compounds emitted by the host tree, Norway spruce (*Picea abies*). Their biological activity is assessed using chemical ecology approaches under field and laboratory conditions, with particular emphasis on their potential application in integrated pest management of *I. typographus*.

The first objective is to determine whether newly infested Norway spruce trees emit elevated amounts of host volatiles during the early stages of bark beetle attack. Special attention is given to *α*-pinene as a model host volatile and potential early indicator of infestation. These objective addresses whether changes in volatile emissions during the first weeks of attack could support the future development of VOC-based sensor systems for early bark beetle detection.

The second objective is to evaluate the biological activity of selected oxygenated monoterpenes (MT-Ox) and estragole, compounds associated with ophiostomatoid fungal metabolism and stress-related changes in host trees. Their effects on *I. typographus* behavior are tested at different doses and in combination with aggregation pheromones in field trapping experiments across several forest locations. Particular focus is placed on two compounds with contrasting effects: 1,8-cineole, acting as an inhibitor of pheromone-mediated attraction, and (+)-isopinocamphone, acting as a synergist that enhances pheromone attraction.

The third objective is to determine whether behavioural and physiological responses to 1,8-cineole and (+)-isopinocamphone differ among beetles according to female body

size. This objective examines field attraction and antennal sensitivity measured by the electroantennography of larger and smaller females to compounds with contrasting biological activity. By linking body size to semiochemical perception and behavioural responses, this part of the thesis aims to clarify the mechanisms underlying female host-selection behavior and individual variation in responses to host-associated volatiles.

The final objective is to synthesize the ecological and applied significance of the identified bioactive semiochemicals. The thesis evaluates how these compounds can contribute to greener, more selective, and more efficient tools for monitoring, early warning, and IPM-based control of *I. typographus*, particularly under the increasing risk of bark beetle outbreaks driven by climate change.

3. Literature review

3.1. Climate-driven forest insect disturbances: integrative monitoring, cataloging, and management strategies

In recent decades, forests worldwide have faced increasing pressure from pest insect outbreaks, particularly in conifer-dominated systems. Wood-boring insects are among the most damaging groups because they colonize internal tree tissues and can rapidly exploit weakened or recently disturbed hosts. Their population dynamics are closely linked to climate change and disturbance regimes, which can alter host susceptibility, breeding-material availability, and insect development rates (Hlásny et al., 2021). Drought stress reduces tree physiological performance and weakens resin-based defenses, thereby lowering host resistance to colonization (Netherer et al., 2019; Netherer and Hammerbacher 2022). Storms and windthrow further increase outbreak risk by generating weakened or fallen spruce material suitable for beetle reproduction (Wermelinger 2004; Netherer et al., 2024). At the same time, warmer conditions can accelerate beetle development and increase voltinism, defined as the number of generations completed per year (Kausrud et al., 2012; Økland et al., 2015). These interacting pressures highlight the need for integrative systems that monitor, catalogue, and compare biotic forest disturbances across regions.

3.1.2. Integrative systems of biotic forest disturbances

Recent efforts to characterize and integrate biotic forest disturbance data led to the development of the Database of European Forest Insect and Disease Disturbances, DEFID2. DEFID2 is a harmonized geospatial dataset compiling records of forest disturbances caused by insects and diseases across Europe. It includes information on disturbance agents, host tree species, damage symptoms, severity, and spatial location, supporting large-scale analyses of biotic disturbance patterns and ecological impacts.

The first DEFID2 release included records from eight European countries: Czechia, Finland, Italy, Romania, Slovakia, Spain, Sweden, and Switzerland. However, the database is strongly dominated by Czechia, which contributed more than 600,000 records, followed by Slovakia and Switzerland. Overall, DEFID2 covers insect- and disease-related forest disturbances from 1963 to 2021. *Ips typographus* was the most frequently recorded disturbance agent, followed by *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae) and *Tomicus destruens* (Coleoptera: Scolytinae) (Figure 1) (Forzieri et al., 2023).

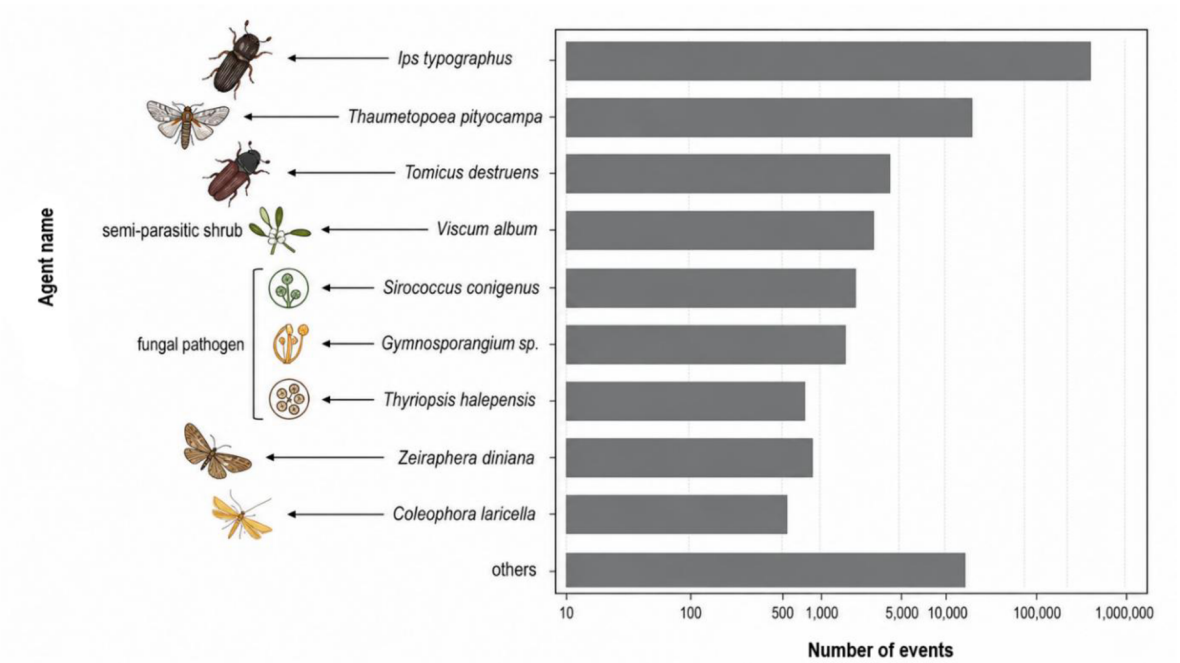

Figure 1. Overview of the primary disturbance agents recorded in DEFID2 during 1963–2021. Bars indicate the number of recorded events for each agent. "Others" represents less frequently reported agents grouped together. Adapted from Forzieri et al. (2023).

3.1.3. General strategies for management and catalogization of forest insect pests

Pest management strategies differ substantially between defoliating moths and bark beetles because these groups vary in feeding behaviour, host-colonization strategy, and exposure to control measures. Defoliating moth larvae feed externally on foliage, buds, or shoots, making them more accessible to biological control agents, including

entomopathogenic fungi (Zúbrik et al., 2021). In contrast, bark beetles such as *I. typographus* feed and reproduce beneath the bark, mainly in the phloem, where they are physically protected from many external treatments. This concealed lifestyle makes bark beetle control more challenging than the management of externally feeding defoliators.

Nevertheless, both external defoliators and subcortical bark beetles contribute to insect-driven forest disturbance. In the Global Forest Resources Assessment (FRA) 2025 dataset, insect-disturbed forest area varied markedly across Europe between 2017 and 2022, with the highest cumulative values reported for Italy, Germany, Czechia, Sweden, and Austria. These records include disturbances caused by defoliators, gallmakers, miners, and borers. At the country level, some areas showed increasing disturbance trends during this period, whereas others, including Italy, Hungary, and Slovakia, declined by 2022. These patterns highlight the spatial and temporal heterogeneity of insect disturbance and the need for pest-specific, regionally adapted forest management strategies (Figure 2).

Country	2017	2018	2019	2020	2021	2022	Mean	Change	Trend
Austria	29.77	42.17	46.96	25.03	22.36	50.01	36.05	20.24	Increase
Czechia	20.70	56.30	67.80	74.70	49.30	30.40	49.87	9.70	Increase
France	1.97	5.73	5.37	5.18	4.19	2.40	4.14	0.43	Increase
Germany	29.35	107.23	119.26	87.06	50.39	50.27	73.93	20.92	Increase
Hungary	6.99	3.11	28.91	20.73	34.82	2.49	16.18	-4.50	Decrease
Italy	110.91	100.86	91.72	83.41	75.85	68.97	88.62	-41.94	Decrease
Slovakia	11.57	10.98	9.45	4.63	4.37	4.94	7.66	-6.63	Decrease
Sweden	31.00	38.00	54.00	61.00	53.00	53.00	48.33	22.00	Increase
Switzerland	7.10	18.69	24.14	15.45	16.89	7.43	14.95	0.33	Increase
Countries in dataset (sum)	**249.36**	**383.07**	**447.61**	**377.19**	**311.17**	**269.91**	**339.72**	**20.55**	**Net increase**

Figure 2. Insect-disturbed forest area in selected European countries, 2017 - 2022. Values are reported in thousand hectares. The data summarizes annual affected area, period mean, 2017 - 2022 change, and trend direction. Data from 2023 and 2024 were not available. Source: FAO Global Forest Resources Assessment 2025; data export © FRA 2026.

3.2. Scolytinae: taxonomy, phenology, bionomy, and fungal symbioses

The Subfamily Scolytinae includes many ecologically and economically important forest pest species. Several genera contain species capable of causing extensive tree mortality during outbreaks, particularly in conifer forests.

Scolytinae (Latreille, 1804) includes approximately 5,800 - 6,050 species worldwide and is commonly divided into bark beetles and ambrosia beetles (Anderson 2002; Hulcr et al., 2015). Ambrosia beetles form an obligate mutualism with fungi, which they cultivate and consume within the xylem, whereas bark beetles are mainly phloeophagous, feeding on nutrient-rich phloem tissues (Hulcr and Stelinski 2017; Hulcr et al., 2015). Scolytines play both beneficial and antagonistic ecological roles: they contribute to wood decomposition and forest succession by colonizing weakened trees, but during outbreaks they can cause severe ecological and socio-economic damage, especially in coniferous forests of North America and Europe (Raffa et al., 2015; Biedermann et al., 2019).

Taxonomically, Scolytinae comprises 26 tribes with a cosmopolitan distribution. Species richness is highest in the Neotropical region, followed by the Oriental, Afrotropical, Palearctic, Nearctic, and Austro-Pacific regions, with approximately 2,260, 1,041, 980, 646, 591, and 448 species, respectively (Hulcr et al., 2015). Morphologically, scolytines are compact, cylindrical beetles, usually 1 - 9 mm long, with a reduced rostrum and geniculate antennae ending in distinct clubs. Their cryptic subcortical lifestyle is supported by adaptations such as sclerotized tibial teeth and apical hooks, or uncus, which aid movement within galleries (Anderson 2002; Jordal 2014).

The evolutionary success of Scolytinae is closely linked to their diverse associations with fungal communities (Hulcr and Stelinski 2017; Tanin et al., 2021). Many species possess specialized integumentary structures, known as mycangia, that transport fungal inoculum (Knížek et al., 2007). For instance, members of Xyleborini, Xyloterini, and Corthylini possess external structures for carrying fungal symbionts, although

these structures vary among tribes (Vega and Hofstetter 2015; Six 2003). In contrast, many Ipini are non-mycangial, with associated fungi carried on the body surface or internally in the gut (Six 2003; Six et al., 2026). These fungal associations may support beetle survival by aiding detoxification of host-tree defenses and producing attractive volatiles that mediate beetle-fungus interactions (Chakraborty et al., 2020a; 2020b; Jirošová et al., 2022; Kandasamy et al., 2023).

The genus *Ips* DeGeer includes some of the most ecologically and economically important scolytine beetles in conifer forests. These species are widely distributed across Europe and are primarily associated with *Picea* spp., although they can also colonize Abies, Larix, Pinus, and Pseudotsuga species (EFSA 2017a).

3.3. *Ips typographus* as an ecologically and economically important Norway spruce pest in Central Europe

"If education alone does not transform society, without it, society cannot change either."
Paulo Freire (1921 - 1997)

Among them, *I. typographus* is the most important species causing large-scale mortality of *Picea abies* during outbreaks (Wermelinger 2004; Hroššo et al., 2020). Because of its economic importance, the biology, ecology, host colonization, and outbreak dynamics of *I. typographus* have been extensively studied. This host-specialized bark beetle is approximately 4.2 - 5.2 mm long and spends most of its life cycle in the subcortical tissues of host trees, mainly *P. abies* (Raffa and Berryman 1983; Biedermann et al., 2019).

3.3.1. Sequence of host-tree colonization by *Ips typographus*

Host colonization is initiated by pioneer males, which locate suitable trees using visual cues and host-derived volatiles (Wermelinger 2004). After penetrating the bark, males excavate a nuptial chamber and release aggregation pheromones, including *cis*-Verbenol (cV) and 2-methyl-3-buten-2-ol, also known as methylbutenol (MT)

(Ramakrishnan et al., 2025). These pheromones attract both males and females, enabling coordinated mass colonization of the host tree (Vega and Hofstetter 2015).

Within the inner bark, *I. typographus* exhibits polygynous reproduction, with one male typically mating with several females in or near the nuptial chamber (Cognato 2015). After mating, females excavate longitudinal maternal galleries and deposit eggs along the gallery walls. A female may lay approximately 60 eggs, although fecundity varies with host quality, temperature, and beetle condition (Austarå and Midtgaard 1986; Anderbrant 1990). Under favorable conditions, eggs hatch after about one week, and larvae develop through successive instars while feeding on the nutrient-rich phloem and cambial tissues (Vega and Hofstetter 2015). At suitable temperatures, development from egg to adult may be completed in 29 - 34 days, allowing rapid population growth during favorable seasons (Wermelinger and Seifert 1998; Doležal and Sehnal 2007). At approximately 30 °C, this life cycle may be shortened to less than 26 days, increasing population growth and contributing to tree mortality during outbreak phases (Wermelinger and Seifert 1998; Hlásny et al., 2021).

Adult flight usually begins in early spring, although swarming depends on habitat temperature and varies with longitude and latitude (Baier et al., 2007; Jakoby et al., 2019). Spring swarming starts after overwintering when temperatures reach approximately 17-20 °C (Lobinger 1994; Ogris et al., 2019). These conditions support flight, maturation feeding, reproduction, host localization, and colonization (Annila 1969; Wermelinger and Seifert 1998, 1999; Doležal and Sehnal 2007; Hofmann et al., 2024). The general stages and consequences of *I. typographus* host colonization are shown below in Figure 3 (A-D).

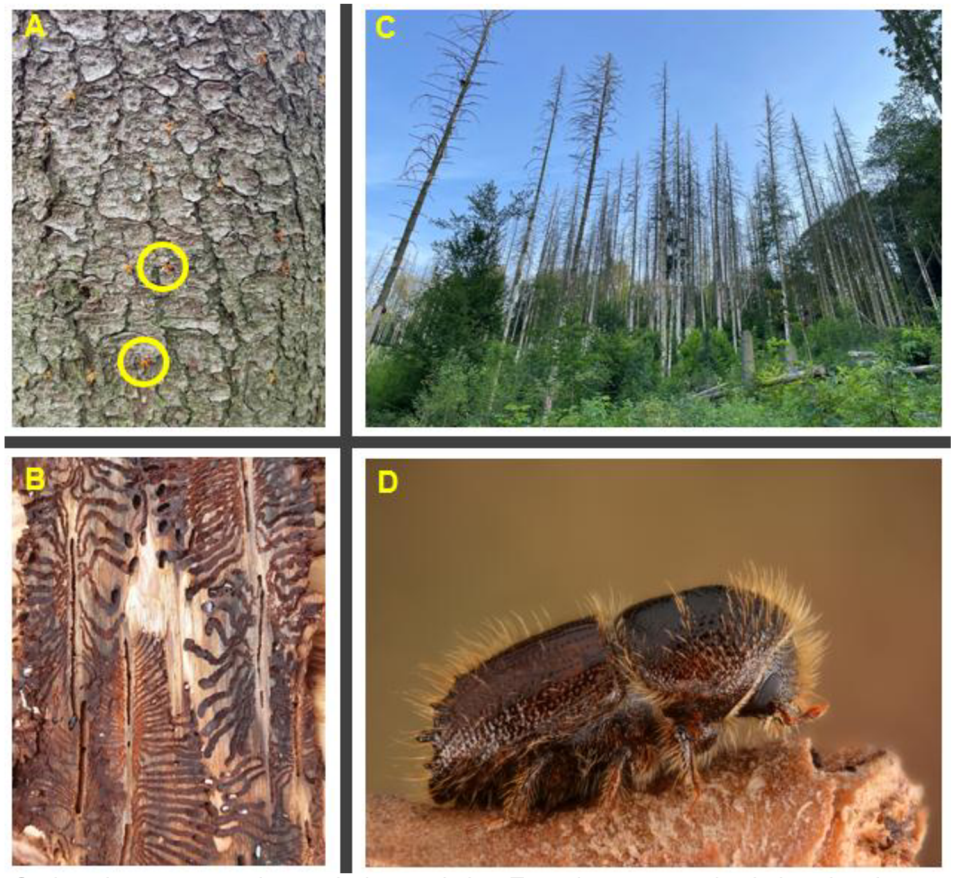

Figure 3. Outbreak stages and morphology of the Eurasian spruce bark beetle, *Ips typographus*. Entrance hole during the colonization phase (e.g., yellow circles) (A); inner bark of an infested Norway spruce tree showing immature stages and adults in galleries (B); host-tree, *P. abies*, mortality following epidemic bark beetle attack (C); adult of *I. typographus* around 5 mm long (D). Image sources: Vladimir Fuka (A), Francesca Marsilli and Filippo Rigodanza (B), B. Phalan (C), and Gilles San Martin (D), accessed through the iNaturalist web portal.

3.3.2. Forest disturbances as climate-change-related drivers of *Ips typographus* outbreaks

In forest stands, the effects of *I. typographus* depend on abiotic and biotic conditions, including host availability, host defense capacity, and beetle population density. These factors influence colonization success and can alter forest structure and composition (Hlásny et al., 2019). Abiotic disturbances, particularly drought, are major drivers of outbreak development because they reduce host vigor and weaken tree defenses (Hlásny et al., 2021b; Netherer et al., 2024). Drought stress can reduce both constitutive and induced defenses, facilitating beetle establishment, reproduction, and

the growth of associated ophiostomatoid fungal symbionts (Basile et al., 2024; Netherer et al., 2024).

Constitutive defenses are continuously present before attack and include preformed toxins, resin ducts, and bark thickness (Wittstock and Gershenzon 2002; Franceschi et al., 2005). In contrast, induced defenses are activated or intensified after herbivore or pathogen attack, allowing trees to conserve resources until protection is needed (Kaplan et al., 2008). In healthy trees, resin ducts store terpenoid-rich toxic compounds that can inhibit bark beetle attacks mechanically, through resin flow, and chemically, through toxic compounds such as 1,8-cineole (Franceschi et al., 2005; Zaman et al., 2025).

Climate change further affects *I. typographus* biology and outbreak dynamics (Seidl and Rammer 2017). Heatwaves and windthrow can weaken host defenses and increase the availability of suitable breeding material (IPPC 2023; Hlásny et al., 2021). Windthrown Norway spruce provides abundant, high-quality breeding substrate for *I. typographus* (Hroššo et al., 2020). Similarly, increasing windthrow frequency and impacts on larger-diameter trees may intensify forest vulnerability to insects and phytopathogens (Štraus and Bončina 2025).

Biotic factors also contribute to outbreak development. High beetle densities can increase tree mortality and reduce wood quality, thereby affecting timber production (Hlásny et al., 2021; Lubojácký et al., 2022). The shift from endemic to epidemic population levels is therefore a central driver of outbreaks (Wermelinger 2004). Secondary bark beetle species may further stress spruce trees by acting as disease vectors and weakening host defenses (MacLean 2016; McTavish et al., 2018; EFSA 2020; Wielkopolan et al., 2021). Although they do not occupy the same ecological niche as *I. typographus*, these species can facilitate its establishment by reducing host resistance (Michel et al., 2024).

3.3.3. Symbiotic fungi of *Ips typographus* as supporting agents for outbreak dynamics

Beyond the direct effects of beetle colonization and phloem feeding, its associated microbial community may influence outbreak dynamics by affecting host colonization, tree defense responses, and beetle performance (Lieutier et al., 2009). These interactions form a functional triad in which fungal symbionts may: (i) degrade or detoxify host defense compounds; (ii) provide nutritional substrates for beetle development; and (iii) produce attractive volatiles that support host localization and aggregation. Together, these processes underline the ecological importance of fungal symbionts in the biology and outbreak potential of *I. typographus* (Zhao et al., 2019; Tanin et al., 2021; Netherer et al., 2021; Lehmanski et al., 2023; Bleiker et al., 2024). Candidate semiochemicals, including (+)-isopinocamphone and fusel alcohols, can mediate interactions from short to long distances, further emphasizing the role of microbial volatiles in bark beetle host selection and communication (Jirošová et al., 2022; Kandasamy et al., 2019, 2023; Moliterno et al., 2023).

Consistent with these functions, several fungal taxa have been associated with conifer-infesting bark beetles, including *I. typographus* (Lieutier et al., 2007; Linnakoski et al., 2012). Across Eurasia, 78 fungal strains have been reported, 40 of them from Europe, mainly from groups commonly associated with bark beetles, especially Ophiostomatales and Microascales. Ophiostomatales include many ophiostomatoid fungi that are frequent bark beetle symbionts, whereas Microascales include saprobic or wood-associated species (Chang et al., 2019). Representative taxa associated with *I. typographus* include *Grosmannia penicillata*, *Leptographium europhioides*, and *Ophiostoma bicolor* within Ophiostomatales, as well as *Endoconidiophora polonica* within Microascales (Kirisits 2007; Linnakoski et al., 2012).

3.3.4. Antagonistic organisms of *Ips typographus*: predators and parasitoids

Although microbial associates may enhance *I. typographus* colonization and performance, its populations are also regulated by antagonistic organisms. Natural

enemies can reduce survival, reproduction, and population growth through predation and parasitism (Wegensteiner et al., 2015). In bark beetle systems, these enemies mainly include predators and parasitoids. Predators kill and consume multiple preys during their lifetime, whereas parasitoids develop on or inside a single host and usually kill it before completing development (Eggleton and Gaston 1990). Bark beetle mortality can be caused by both groups, although their effects vary with beetle density (Moore 1972; Weslien and Regnander 1992; Reeve 1997; Wermelinger 2002).

Predators of bark beetles, including Ips spp., include woodpeckers (Aves: Picidae), beetles (Coleoptera), flies (Diptera), true bugs (Hemiptera: Heteroptera: Anthocoridae), and mites (Acari: Mesostigmata) (Wegensteiner et al., 2015). These predators can act as important natural regulators of bark beetle populations and may help prevent outbreak development (Hlásny et al., 2019). Woodpeckers remove larvae and adults from beneath the bark and may expose concealed galleries to other natural enemies (Wegensteiner et al., 2015). Among arthropods, clerid beetles are among the best-known bark beetle predators. Species such as *Thanasimus formicarius* and *Thanasimus dubius* attack adult bark beetles, including *I. typographus*, on the bark surface, while their larvae feed within galleries (Poland and Borden 1997). Other predatory beetles include Trogossitidae, Histeridae, Monotomidae, and Staphylinidae, while predatory flies, especially *Medetera* spp. (Dolichopodidae), attack bark beetle larvae under the bark (Wegensteiner et al., 2015).

Predators and parasitoids often locate prey using semiochemicals, including bark beetle pheromones, host-tree volatiles, and microbial symbiont volatiles (Raffa and Dahlsten 1995; Poland and Borden 1997; Reeve 1997; Wermelinger 2002; Reeve et al., 2003; Pettersson and Boland 2003; Boone et al., 2014; Moliterno et al., 2023; Sousa et al., 2024). Parasitoids form another important natural-enemy guild in bark beetle communities. Most documented parasitoids associated with Ips species, including *I. typographus*, are wasps, particularly *Rhopalicus* spp. (Hymenoptera: Pteromalidae) and Coeloides spp. (Hymenoptera: Braconidae) (Pettersson et al., 2001a, 2001b;

Pettersson and Boland 2003; Wegensteiner et al., 2015). These parasitoids attack eggs, larvae, or pupae concealed under bark.

The impact of parasitoids depends on host accessibility, bark thickness, host density, phenological synchrony, and forest management (Wegensteiner et al., 2015). Phenological synchrony refers to the match between vulnerable host stages and active natural-enemy stages (Welch and Harwood et al., 2014). Parasitoids may respond rapidly to local increases in bark beetle abundance, especially in fresh windthrown or recently attacked trees, but their effectiveness often depends on prey developmental stage (Wegensteiner et al., 2015). Because parasitoid populations typically increase after host populations have already risen, predators and parasitoids may contribute more to maintaining endemic bark beetle populations than to suppressing large outbreaks once established (Wermelinger 2002; Kenis et al., 2004; Hilszczański et al., 2007; Berisford 2011; Wegensteiner et al., 2015).

3.3.5. The role of behaviourally active volatile compounds in *Ips typographus*

Bark beetle behaviour and ecology are strongly influenced by semiochemical-mediated multitrophic interactions. These intricate chemical interactions involve species-specific bark beetle compounds, host-tree volatiles, metabolites produced by symbiotic fungi, and chemical cues associated with competitors, predators, and parasitoids (Wermelinger et al., 2012; Tanin et al., 2021; Netherer 2022; Fettig et al., 2025). Behaviourally active compounds are central to the chemical ecology of *I. typographus* and underpin semiochemical approaches for monitoring and management. Here, the term refers to volatile chemicals that modify *I. typographus* behaviour. These compounds may originate from the beetle through de novo biosynthesis, its host tree, associated fungi, or non-host plants.

3.3.5.1. Aggregation pheromone

The aggregation pheromone of *I. typographus* is mainly composed of 2-methyl-3-buten-2-ol and *cis*-Verbenol, while ipsdienol may occur as a minor component in some

populations or blends. One reported blend contained 2-methyl-3-buten-2-ol, *cis*-Verbenol, and ipsdienol at approximately 9:1:0.1, respectively (Ramakrishnan et al., 2025). However, attraction is strongly reduced or lost when either 2-methyl-3-buten-2-ol or *cis*-Verbenol is removed (Schlyter et al., 1987). In synthetic pheromone traps used in field experiments, ipsdienol is often excluded, and lures commonly follow the approximate 10:1 ratio observed in natural emissions (Jirošová et al., 2022; Moliterno et al., 2023). Commercial pheromone baits may also differ in qualitative and quantitative composition among manufacturers (Šramel et al., 2021; Heber et al., 2021).

Other male hindgut compounds may contribute to the pheromone bouquet, including trans-Verbenol, myrtenol, *trans*-myrtanol, and 2-phenylethanol. However, unlike 2-methyl-3-buten-2-ol and *cis*-Verbenol, these compounds are not consistently required for attraction (Schlyter et al., 1987; Francke et al., 1995; Sun et al., 2006). Although ipsdienol is produced in minor quantities by males of *I. typographus*, its behavioural role may depend on enantiomeric composition, which varies among populations. Such stereochemical variation can influence species recognition and may reduce cross-attraction among sympatric bark beetle species (Ramakrishnan et al., 2025). Aggregation pheromone production and composition also vary with adult life stage, mating status, and boring activity into host trees (Schlyter and Löfqvist 1986; Sun et al., 2006; Ramakrishnan et al., 2022). During later attack stages, verbenone may increase as host resources decline. It is often interpreted as an anti-aggregation cue, although its behavioural effect in *I. typographus* depends on dose and release rate (Frühbrodt et al., 2024a).

3.3.5.2. Host compounds

Electrophysiological studies show that *I. typographus* detects several host-related oxygenated monoterpenes through antennal sensilla (Andersson et al., 2009; Kalinová et al., 2014; Schiebe et al., 2019; Hou et al., 2021). Host volatile organic compounds also guide host location and assessment. Norway spruce emits monoterpenes such as

α-pinene, *β*-pinene, limonene, myrcene, and *β*-phellandrene, as well as oxygenated monoterpenes such as 1,8-cineole and phenolic compounds such as estragole (Netherer et al., 2021). These volatiles can indicate host identity, physiological stress, and colonization suitability. Although oxygenated monoterpenes often occur at low concentrations, they can strongly affect beetle olfaction and behaviour. Relevant examples include 1,8-cineole, (+)-isopinocamphone, estragole, pinocamphone, α-terpineol, *α*-pinene, camphor, carvone, terpinen-4-ol, and (+)-*trans*-4-thujanol (Kalinová et al., 2014; Netherer et al., 2021; Jirošová et al., 2022; Moliterno et al., 2023). These compounds may act as long- and short-range cues for host assessment, particularly in stressed, felled, or fungus-colonized spruce tissues (Schiebe et al., 2019; Lehmanski et al., 2023).

The behavioural effects of host volatiles depend on dose, release rate, blend composition, and environmental conditions. For example, host monoterpenes may enhance attraction when combined with aggregation pheromones, whereas high concentrations may reduce attraction, feeding, or host acceptance. Some oxygenated monoterpenes, including 1,8-cineole (Andersson et al., 2010; Jirošová et al., 2022) and (+)-*trans*-4-thujanol, reduce attraction in dose-dependent field experiments (Jirošová et al., 2022; Moliterno et al., 2023). In contrast, (+)-isopinocamphone can elicit strong field responses in *I. typographus*. Interestingly, associated predators and parasitoids also respond to 1,8-cineole and (+)-isopinocamphone (Moliterno et al., 2023). These patterns highlight the importance of blend-dependent signals involving pheromones and host-tree compounds, rather than compounds acting as universally attractive or repellent cues. As in other bark beetles, molecular configuration is also fundamental to behavioural activity in *I. typographus*, with changes in stereoisomer composition influencing behavioural responses (Vité 1978; Mori 2007; Jirošová et al., 2022). The figure 4 illustrates chemical chirality, or mirror-image molecular conformation, in relation to *I. typographus* behavioural activity.

Phenolic compounds also influence beetle-tree-fungus interactions. In Norway spruce, phenolics contribute to defense against oxidative stress, drought stress, and fungal

invasion (Franceschi et al., 2005; Six et al., 2026). Feeding assays indicate that phenolic compounds can reduce feeding or tunnelling by *I. typographus*, although their effects vary among compounds and concentrations (Faccoli and Schlyter 2007). At high doses, estragole reduces attraction to pheromone-baited traps (Moliterno et al., 2023). Some fungal associates can metabolize spruce phenolics, potentially weakening host defenses and improving the substrate for larval development (Wadke et al., 2016; Zhao et al., 2019).

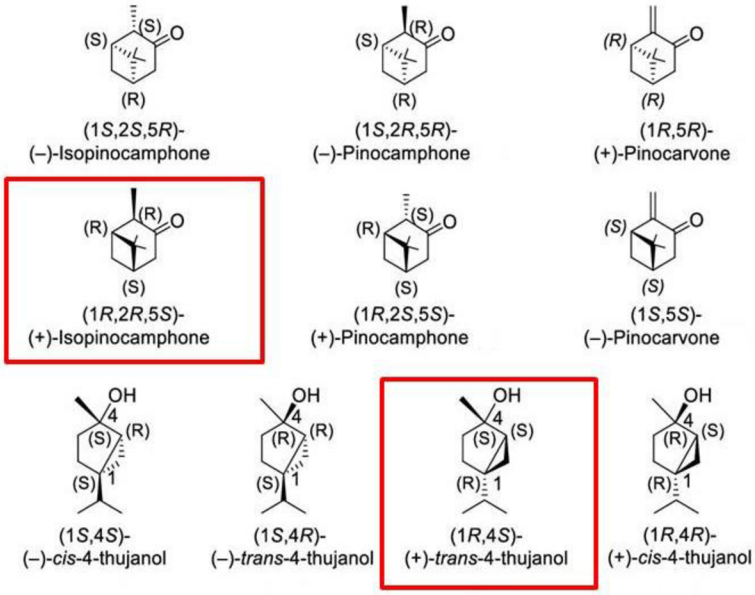

Figure 4. Examples of semiochemical structures showing the absolute configuration of synthesized compounds belonging to the oxygenated terpenoid group. Enantiomers with behavioural relevance to *I. typographus* are shown in red. *R* and *S* indicate stereocentres labelled according to optical rotation: *R* = "Rectus," from Latin meaning "right," and *S* = "Sinister," from Latin meaning "left." Nomenclature follows IUPAC recommendations (Leigh 2011). Marked compounds were tested in field experiments (Jirošová et al., 2022; Moliterno et al., 2023). Figure adapted from Ganji et al. (2020).

3.3.5.3. Non-host volatiles

Non-host volatiles are another important group of behaviourally active compounds. These odours are released by unsuitable host plants, particularly angiosperm trees such as birch. In mixed forest landscapes, non-host volatiles may help *I. typographus* avoid unsuitable hosts. Important non-host cues include green leaf volatiles, C8 alcohols, and trans-conophthorin. These compounds can interrupt attraction to aggregation pheromones and reduce responses to pheromone-baited traps (Zhang

2003; Zhang and Schlyter 2003, 2004; Unelius et al., 2014; Jakuš et al., 2024). Non-host volatiles therefore contribute to host discrimination and are promising candidates for Push-Pull management strategies (Jakuš et al., 2022).

3.3.5.4. Fungal volatiles

Fungal volatiles add a microbial dimension to the semiochemical ecology of *I. typographus*. The beetle is associated with ophiostomatoid fungi, including taxa related to *Ophiostoma* sp. and *Grosmannia* sp. (Kandasamy et al., 2016). These fungi colonize the phloem and sapwood and can modify the chemical environment within attacked trees. Through fungal metabolism, host monoterpenes can be transformed into volatile metabolites detectable by beetles (Zhao et al., 2019; Netherer et al., 2021). Recent evidence suggests that *I. typographus* can use fungal metabolites as cues for locating fungal symbionts and suitable colonization substrates (Kandasamy et al., 2023). Host selection is therefore shaped not only by beetle pheromones and tree volatiles, but also by microbial activity within the host substrate (Raffa 2001; Schiebe et al., 2019; Netherer et al., 2021), as shown in Figure 5.

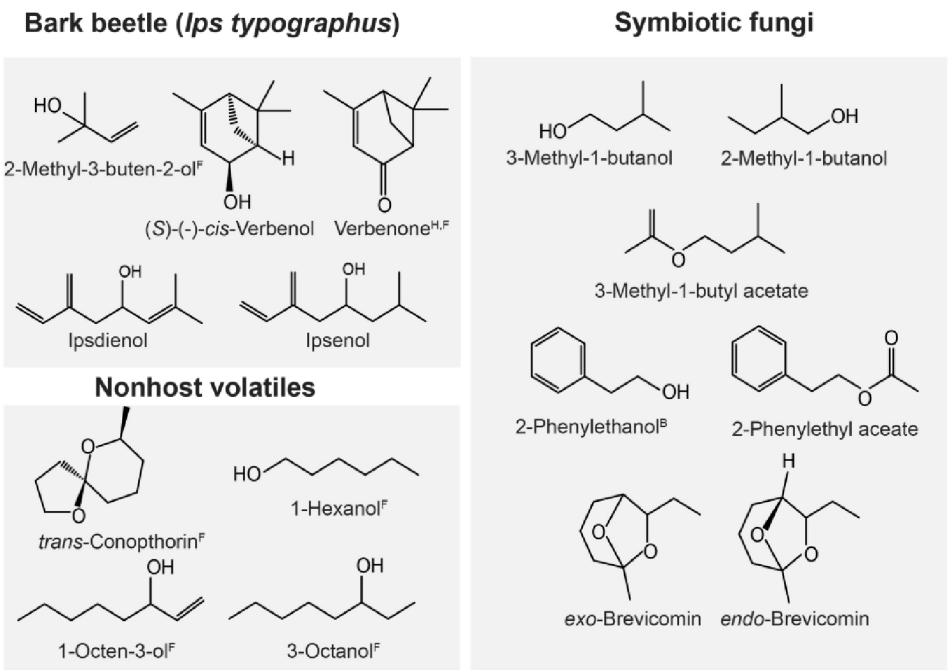

Figure 5. Illustration of volatile semiochemical structures and qualitative diversity in intra- and interspecific interactions among *I. typographus*, its host tree *Picea abies*, other bark beetles, and fungal symbionts. Semiochemical origins are indicated by suffixes next to compound names: H = host tree, B = beetle, and F = fungus. Source adapted from Netherer et al. (2021).

3.3.5.5. Detection system, olfaction, and navigation

As shown above, ecological and behavioural responses in *I. typographus* depend on its olfactory system. Odour molecules are detected by olfactory sensory neurons (OSN) housed in antennal sensilla (Andersson et al., 2010; Andersson 2012). These neurons express chemoreceptors that convert chemical information into neural activity and, ultimately, behavioural responses. This sensory system allows the beetle to integrate pheromones, spruce volatiles, non-host odours, fungal metabolites, and signals from competitors or natural enemies (Kalinová et al., 2014; Schiebe et al., 2019; Shewale et al., 2025; Ramakrishnan et al., 2025). Together, these findings show that behaviourally active volatile compounds in *I. typographus* form an integrated chemical information system. Aggregation pheromones coordinate mass attack, host volatiles indicate host identity, stress status, and defensive condition, non-host volatiles reduce attraction to unsuitable plants, and fungal volatiles signal microbial colonization and substrate quality (Netherer et al., 2021; Jirošová et al., 2022). Phenolics and oxygenated monoterpenes further influence feeding, attraction, repellence, and host acceptance in bark beetles (Hayes and Strom 1994; Schiebe et al., 2019).

At the population level, field experiments in Central Europe show that high doses of 1,8-cineole and (+)-*trans*-4-thujanol, when combined with pheromone traps, reduce total catches and significantly affect females of *I. typographus* (Jirošová 2022; Moliterno et al., 2023). Because males are the pioneer sex, producing long-range aggregation pheromones and exhibiting polygynous behaviour (Schebeck et al., 2023), females are central to reproduction and fitness through their roles in host-tree selection and mate assessment (Schlyter and Zhang 1996; Paynter et al. 1990). Raffa (2001) described the complexity of bark beetle ecological interactions under abiotic and biotic pressures, including evolutionary trade-offs, predation risk, pheromone adaptation, and tritrophic and intraspecific interactions. In odour-rich environments, semiochemical diversity may

impose selective pressures on olfactory systems, as suggested by VOC *β*-diversity patterns reflecting structural heterogeneity in vertical habitat strata (Schlyter et al. 2015; Hou et al., 2021; Lehmanski et al., 2023; Six et al., 2025; Carlson et al., 2025 preprint.). Laboratory studies further show that olfactory variation in *I. typographus* occurs at the molecular level. For example, the odorant receptor ItypOR33 and its variant ItypOR33a may differ qualitatively in their responses to Ips spp. pheromone compounds, such as amitinol and *S*-(-)-ipsenol, respectively (Johny et al., 2023). In the field, host-tree health strongly influences beetle acceptance and colonization (Netherer et al., 2021). Healthy host trees may deter severe infestation and affect *I. typographus* brood fitness, including body size (Grodzki 2004; Sallé et al., 2005). Intriguingly, females with different body sizes show distinct antennal and behavioural responses in pheromone traps baited with 1,8-cineole and (+)-isopinocamphone. Smaller females showed unequal antennal and behavioural responses to 1,8-cineole, suggesting that central olfactory processing may help them select less competitive or lower-quality hosts. Larger females detected (+)-isopinocamphone more effectively, which may improve host-tree selection. These results link beetle phenotype with olfactory behaviour and host-use strategies (Moliterno et al., 2025).

Research on behaviourally active compounds has direct relevance for management because it supports the development of more ecologically realistic semiochemical blends. Effective monitoring and management should consider blend composition, release rates, stereochemistry, beetle density, host condition, and field context. Testing candidate compounds under realistic field conditions is therefore essential for improving pheromone-based monitoring, semiochemical management of *I. typographus* outbreaks, and the development of ecological bioinputs under climate change.

3.3.5.6. Management strategies for Ips typographus outbreaks

Field management of *I. typographus* outbreaks requires an integrated framework that considers both abiotic and biotic pressures, highlighting the need to combine

prevention, monitoring, and management (Wermelinger 2004; EFSA 2017; Bozzini et al., 2026). Prevention aims to reduce stand susceptibility through diversified forest composition, reduced tree stress, and rapid removal of fresh windthrow before beetle reproduction (Wermelinger 2004; Lindmark et al., 2022; Romeiro et al., 2024; Bozzini et al., 2026). Monitoring relies on field inspections, pheromone-baited traps, and risk models to estimate beetle activity, phenology, developmental timing, and likely damage (Weslien et al., 1989; Wermelinger 2004; Romeiro et al., 2024; Blake et al., 2024).

Management actions include suppression and recovery measures. Sanitation felling is a key suppression strategy involving the cutting and removal of infested trees before new adults emerge from the bark (Wermelinger 2004; Miścicki and Grodzki 2021; Ait et al., 2025; Bozzini et al., 2026). In contrast, salvage logging removes freshly windthrown, broken, or damaged spruce before colonization, thereby reducing available breeding material. Thus, sanitation felling targets already colonized trees, whereas salvage logging targets damaged but not yet colonized material (Wermelinger 2004; Ait et al., 2025). Trap logs are another suppression tool: freshly cut logs release attractive chemical cues that lure beetles (Kalinová et al., 2014), but they must be carefully monitored and removed or debarked before mature beetles emerge (Lubojacký and Holuša 2011; Lindmark et al., 2022; Ait et al., 2025). Trap logs may also be combined with synthetic insecticides in attract-and-kill systems (EPPO 2026). However, their effectiveness can decline during severe outbreak peaks, and insecticide-based approaches raise concerns about resistance, non-target effects, and broader environmental impacts (Lubojacký and Holuša 2011; Heber et al., 2021; Bracalini et al., 2025; Kula and Hrdlička 2025).

Although sanitation felling and salvage logging remain among the most widely recommended bark beetle management strategies (Stadelmann et al., 2013; Hlásny et al., 2019; Bozzini et al., 2026), they also carry major operational demands. In the Czech Republic, wood removals reached 33.3 million m^3 in 2021, equivalent to almost 3.11 m^3 per person (UNECE 2023). This illustrates the scale of sanitation- and salvage-

based responses, including costs for harvesting, extraction, transport, and storage, even when rapidly harvested timber retains commercial value (Fernholz et al., 2020).

Bark beetle management must also align with broader environmental policy frameworks. The European Green Deal, launched by the European Commission in 2019, supports the transition toward climate neutrality and contributes to the United Nations Sustainable Development Goals. In forest protection, bark beetle management is especially relevant to SDG 15, Life on Land, which emphasizes sustainable forest management, and SDG 13, Climate Action, which supports climate-adaptive ecosystem protection. Semiochemical-based strategies are consistent with these goals because they provide targeted and ecologically compatible options for reducing bark beetle pressure while minimizing chemical inputs and non-target impacts (Bergström, 2007; Seybold et al., 2018). This is particularly important because interacting disturbances, such as drought and bark beetle outbreaks, can increase susceptibility to catastrophic wildfires and compromise entire ecosystems (Köhl et al., 2024; Beetz et al., 2025).

3.3.5.7. Use of pheromones and other semiochemicals as integrated tools in Ips typographus management

Within this sustainability framework, pheromone-based monitoring and control are important tools for ecologically oriented bark beetle management. Pheromone traps use synthetic aggregation pheromones, which mimic naturally, produced chemical signals, to monitor beetle flight activity and, in some cases, reduce local population density through mass trapping (Weslien et al., 1989; Heber et al., 2021; Kuhn et al., 2022; Ramakrishnan et al., 2025). Although environmentally compatible for *I. typographus* management, their effectiveness varies across landscapes and populations. Field experiments show dose-dependent responses to pheromone-baited traps, indicating that lure composition, release rate, trap density, and spatial arrangement strongly influence trapping success (Schlyter and Löfqvist 1986; Schlyter et al., 1987; Franklin and Grégoire 2001; Moliterno et al., 2023). Untreated or inaccessible forest areas may also act as refuges, allowing beetles to persist and recolonize surrounding stands. Therefore, pheromone mass trapping should not be

considered a stand-alone method during severe outbreaks, particularly when long-distance dispersal, migration, and high regional population pressure weaken local control efforts (Wermelinger 2004; EFSA 2017; Kuhn et al., 2022; Romeiro et al., 2024; Bracalini et al., 2025).

Pheromones are versatile because they can be integrated into push-pull systems that use chemical signals to modify insect behaviour, including aggregation pheromones, host volatiles, non-host volatiles, and anti-attractants (Zhang and Schlyter 2003, 2004; Cook et al., 2007; Ramakrishnan et al., 2025). The pull component usually relies on synthetic aggregation pheromones placed in traps, on trap logs, or near bait trees to intercept beetles before they attack protected spruce stands (Wermelinger 2004; Lindmark et al., 2022; Jakuš et al., 2022; Korolyova et al., 2026). The push component uses anti-attractants that reduce beetle orientation to protected trees by repelling beetles or masking attractive odour cues through the olfactory system (Bakke 1981; Zhang and Schlyter 2003; Andersson et al., 2010). Non-host volatiles, especially odours released by unsuitable angiosperm hosts, can also reduce attraction to spruce-associated cues (Zhang and Schlyter 2003, 2004; Schiebe et al., 2011; Unelius et al., 2014).

Technical and logistical implementation is critical for push-pull success. Systems may fail when the attractive pull is stronger than the available interception capacity. Trap density, meaning the number of traps per treated area, must be sufficient because low density can attract beetles without capturing enough individuals to prevent nearby attacks (Byers 2007; Cook et al., 2007; Kuhn et al., 2022; Jakuš et al., 2022; Korolyova et al., 2026). Spillover occurs when pheromone lures increase local beetle arrival, but traps fail to intercept enough individuals, increasing attacks near traps or forest edges (Kuhn et al., 2022; Korolyova et al., 2026). Switch effects occur when anti-attractants redirect attacks from treated trees to adjacent untreated trees (Jakuš et al., 2022; Jakuš et al., 2024). Effective *I. typographus* management therefore requires coordinated lure placement, trap density, dispenser spacing, sanitation felling, and trap maintenance aligned with local beetle pressure and seasonal flight timing (Wermelinger 2004; Jakuš

et al., 2022; Deganutti et al., 2024; Hallas et al., 2026), while also considering forest ecology, beetle biology, and climate-related conditions (Raffa 2001; Stříbrská et al., 2022, 2024; Netherer et al., 2024).

3.3.5.8. Semiochemical mediated interactions with natural enemies

Volatile signals released during beetle colonization can also be used by natural enemies. Parasitoids, including pteromalid and braconid wasps, and predators such as *Thanasimus* spp. (Coleoptera: Cleridae), use beetle-associated volatiles to locate hosts or prey (Wegensteiner et al., 2015). Natural enemies of *I. typographus* may detect trace pheromone components or specific isomeric blends as kairomonal cues (Tømmerås 1985), while host-tree volatiles also contribute to these multitrophic interactions. Field tests showed that *Thanasimus dubius* (F.) is attracted to (-)-*α*-pinene (Erbilgin and Raffa 2001; Hulcr et al., 2006). The bicyclic monoterpenoid isopinocamphone was detected by electroantennography in *Rhopalicus tutela* Walker (Hymenoptera: Pteromalidae) and *Coeloides bostrichorum* Giraud (Hymenoptera: Braconidae) (Pettersson et al., 2001a, 2001b), and pheromone traps baited with host-tree volatiles, including (+)-isopinocamphone, attracted parasitoid wasps (Moliterno et al., 2023). In addition, the attraction of natural enemies related to the long-legged predator *Medetera* species (Diptera: Dolichopodidae) (Sousa et al., 2023, 2024) were also evidenced.

3.3.5.9. Policy of Ips typographus management

Beyond the bioecology and outbreak dynamics of *I. typographus*, policy frameworks play a central role in shaping responses to biotic and abiotic forest disturbances. According to the FRA 2025 database, countries bordering the Czech Republic, Austria, Germany, Hungary, Poland, and Slovakia and all report national policies and legislation supporting sustainable forest management (SFM), indicating that disturbance responses are embedded within broader governance systems. These policies influence monitoring, sanitary interventions, salvage logging, restoration, biodiversity protection, and climate-adaptation measures. However, management capacity and implementation may differ among countries because of contrasting ownership

structures and institutions. For example, Austria is dominated by private forest ownership, whereas Poland has a predominantly public forest estate, which may affect the coordination, speed, and scale of responses to insect outbreaks, drought, windthrow, and fire. Based on the FRA database, only Poland did not report promoting or allowing stakeholder participation in forest policy development, while only Germany did not report a traceability system for wood products. Nevertheless, both countries support SFM through long-term frameworks balancing the economic, social, and environmental values of forests.

The implication is that disturbance management cannot be understood solely as a biological or technical issue. It also depends on policy design, land ownership, stakeholder participation, and the balance between production, protection, and conservation objectives. Under climate change, these governance differences may become increasingly important because interacting disturbances, including drought, windthrow, bark beetle outbreaks, and wildfire, can differ among countries and require coordinated, adaptive landscape-scale management aligned with investment in science and education.

3.3.5.10. Other Ips species feeding on Norway spruce

Several Ips species also feed on *P. abies*. Norway spruce is considered a major host for *I. duplicatus* and *I. amitinus*, whereas for *I. cembrae* and *I. sexdentatus* it is regarded as a secondary or occasional host (EFSA 2026). *Ips duplicatus* and *I. amitinus* have been reported feeding in the upper trunk of *P. abies* and showing phenological patterns similar to *I. typographus* (Holuša et al., 2012; EFSA 2017b; EFSA 2026). However, during epidemic population phases, feeding activity may extend across the vertical strata of the host tree, from the lower trunk to the canopy (Ramakrishnan et al., 2025). Attack phenology may also vary with forest structure, as natural forest edges can provide warmer and more attractive microclimatic conditions for beetle swarming than inner forest stands (Stříbrská et al., 2022).

Among the major threats to conifers such as *P. abies*, *I. typographus* remains especially important, as highlighted by the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests, ICP Forests. In 2023, *P. abies* showed a mortality rate of 1.5%, with 156 newly dead trees recorded. This exceeded the overall tree mortality rate of 1.1%, indicating comparatively elevated mortality in *P. abies* relative to several other main tree species groups (Figure 6) (Michel et al., 2024).

Main species or species groups	N dead trees	Mortality rate (%)	Main specific cause of death
Scots pine (*Pinus sylvestris*)	171	1.0	Fire
Norway spruce (*Picea abies*)	156	1.5	*Ips typographus*
Austrian pine (*Pinus nigra*)	42	0.8	Fire
Mediterranean lowland pines	43	0.5	Drought
Other conifers	49	0.6	
Common beech (*Fagus sylvatica*)	69	0.6	Various fungi
Deciduous temperate oaks	112	1.3	Wind
Dec. (sub-) Mediterranean oaks	78	1.0	Fire & drought
Evergreen oaks	43	0.9	Fire & drought
Other broadleaves	401	2.0	
Total			
Conifers	461	0.9	
Broadleaves	703	1.3	
All species	**1 164**	**1.1**	

Figure 6. Number of dead trees, mortality rates, and main identifiable causes of death recorded in the field for the main tree species and species groups in 2023. ICP Forests Assessment 2024. Source: Michel et al. (2024).

3.3.5.11. Defoliators as other spruce pests

In addition to bark beetles and other biotic disturbances that may influence *I. typographus* outbreaks, defoliating insects represent an important pest guild in coniferous forests (FISE 2025). This group includes polyphagous species such as *Lymantria dispar* (L.), the gypsy moth, Lepidoptera: Erebidae, and *Diprion pini* (L.), a sawfly, Hymenoptera: Diprionidae (La Porta et al., 2008; Zúbrik et al., 2021). Several defoliating moths, including *Epirrita autumnata* (Borkhausen), *Operophtera brumata* (L.), and *Erannis defoliaria* (Clerck) (Lepidoptera: Geometridae), are also polyphagous, and climate change may contribute to their transition into outbreak or epidemic phases (Fält-Nardmann et al., 2016, 2018).

In Norway spruce forests of Czechia, defoliation pressure is associated with a diverse assemblage of Lepidoptera that use *P. abies* as a food resource. Modlinger and ka (2016) documented 96 Lepidoptera species, including 69.8% for which spruce is a usual host, indicating their relevance as biotic stress agents in Czech spruce forests. This importance is further increased by the dominance of Norway spruce, which represents 49.50% of planted tree species and approximately 477.60 million m^3 of forest volume in the Czech Republic (FAO 2025).

4. Materials and Methods

This section describes the laboratory and field experiments, the overall experimental framework, and the statistical analyses associated with the three publications included in this dissertation (I, II and III).

In *Publication I*, laboratory and field experiments were conducted to optimize the collection of *α*-pinene and other volatile organic compounds (VOCs) released from spruce logs simulating bark beetle attack.

Publications II and *III* focused on evaluating the responses of *Ips typographus* to synthetic volatile compounds used in combination with pheromone-baited traps. In *Publication II*, estragole, 1,8-cineole, (±)-camphor, (−)-carvone, *α*-terpineol, (−)-terpinen-4-ol, (+)-pinocamphone, and (+)-isopinocamphone were tested at low, medium, and high release rates at two field sites in the Czech Republic. *Publication III* further examined the effects of 1,8-cineole and (+)-pinocamphone under field conditions.

4.1. Laboratory and Field Experiments and Statistical Analysis: Publication I

I. Stříbrská, B., Moliterno, A. A. C., Hüttnerová, T., Leiner, M., Surový, P., & Jirošová, A. (2024). Pilot study of the 3D spatial distribution of α-pinene emitted by Norway spruce, *Picea abies* (L.) Karst., recently infested by *Ips typographus* (L. 1758) (Coleoptera: Scolytinae). Forests, 15(1), 10. https://doi.org/10.3390/f15010010

- Laboratory experiment

During the laboratory phase, freshly cut *Picea abies* logs were maintained under controlled conditions at approximately 25 °C and 60% relative humidity. Bark beetle attack was simulated by drilling small holes through the bark and into the phloem. Volatile organic compounds (VOCs) were subsequently collected using solid-phase microextraction (SPME) fibers positioned at different heights and distances from the logs. This experimental arrangement enabled an assessment of how *α*-pinene abundance varied spatially around the simulated infestation sites (Figure 7).

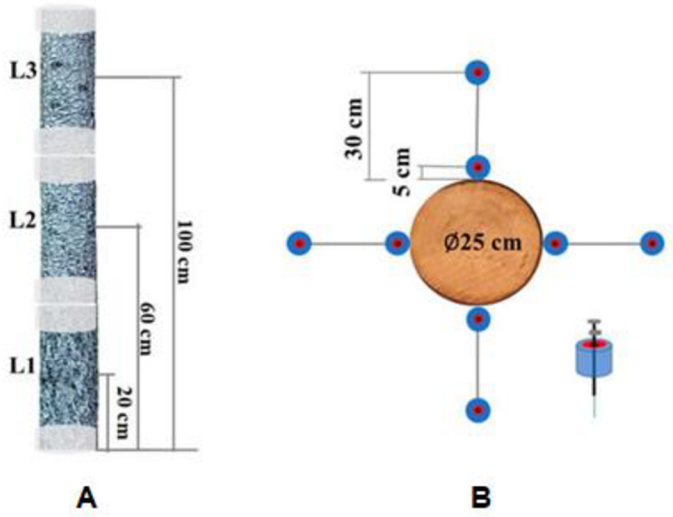

Figure 7. Methodology used in *Publication I* for collecting *α*-pinene under laboratory conditions from freshly drilled *Picea abies* logs. A: *α*-Pinene was collected at three height levels, designated L1, L2, and L3. B: Top view of the log pile. Black dots indicate the distances between the bark surface and the *α*-pinene sampling points at each height level.

- Field experiment

During the field phase, VOCs were sampled around two Norway spruce trees naturally infested by *Ips typographus* at forest sites in the Czech Republic during the 2022 growing season. The trees were classified as recently infested based on the presence of frass at the base of the stem and were estimated to have been under attack for approximately two weeks. Sampling points were established at three heights above

ground - 1.3, 2.6, and 4.0 m - and at three distances from the trunk - 5, 100, and 900 cm (Figure 8).

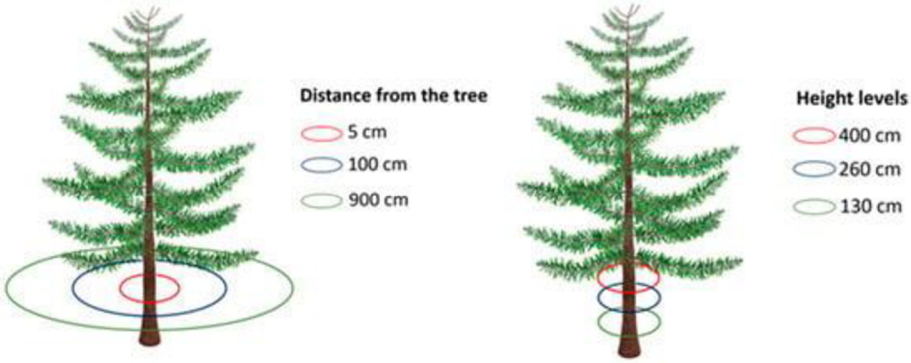

Figure 8. Sampling directions and positions established for VOC collection around Norway spruce trees recently infested by *Ips typographus*.

Samples were collected in the four cardinal directions: north, east, south, and west. Environmental variables, including air temperature, relative humidity, wind speed, and wind direction, were recorded during sampling. Two study sites were selected: A) Stříbrná Skalice, Czech Republic (49.913° N, 14.863° E), and B) the Czech University of Life Sciences Prague forests near Vyžlovka, Czech Republic (49.981° N, 14.801° E) (Figure 9A, B).

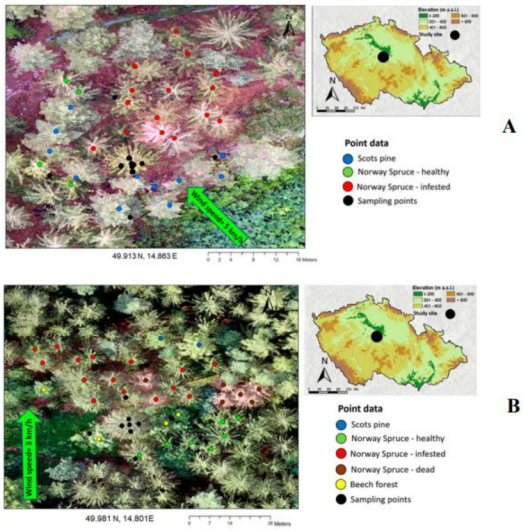

Figure 9. Study sites used for spatial measurements of VOCs around infested trees in the Czech Republic. A: Stříbrná Skalice site, sampled on 30 June 2022. Black symbols represent sampling points; red, infested or dead Norway spruce; green, healthy Norway spruce; and blue, Scots pine. B: Vyžlovka site, sampled on 24 August 2022. Black symbols represent sampling points; red, infested Norway spruce; green, healthy Norway spruce; brown, dead Norway spruce; blue, Scots pine; and yellow, European beech.

- VOC collection and chemical analysis

Two VOC collection methods were compared. The first method involved exposing SPME fibers to forest air for 60 min, after which the fibers were stored under refrigerated conditions until chemical analysis. The second method used HayeSep-Q® sorbent cartridges connected to calibrated air-sampling pumps. Air was drawn through each cartridge at a flow rate of 100 mL min^{-1} for 60 min.

Chemical analyses were performed using gas chromatography coupled with flame ionization detection (GC-FID). The analysis focused on *α*-pinene because it was the dominant monoterpene detected and was therefore considered representative of the broader monoterpene emission pattern. Results obtained using SPME fibers were expressed as relative *α*-pinene peak areas, whereas samples collected using sorbent cartridges were quantified using a calibration curve prepared from *α*-pinene standards.

- Statistical analysis

Data normality was assessed using the Shapiro-Wilk test. Because the data did not satisfy the assumption of normality, differences in *α*-pinene abundance among sampling distances, heights, and cardinal directions were evaluated using nonparametric Kruskal-Wallis tests. Where significant differences were detected, appropriate post hoc multiple-comparison tests were applied to identify differences among individual groups.

4.2. Laboratory and Field Experiments and Statistical Analysis: Publications II and III

II. Moliterno, A. A. C., Jakuš, R., Modlinger, R., Unelius, C. R., Schlyter, F., & Jirošová, A. (2023). Field effects of oxygenated monoterpenes and estragole combined with pheromone on the attraction of *Ips typographus* and its natural

enemies. Frontiers in Forests and Global Change, 6, 1292581. https://doi.org/10.3389/ffgc.2023.1292581

- Laboratory preparation and dispenser calibration

Publication II included the laboratory preparation and calibration of odour dispensers for field-trapping experiments designed to evaluate the responses of *Ips typographus* and its natural enemies. Dispensers were prepared for eight synthetic host-associated compounds: estragole, 1,8-cineole, (±)-camphor, (-)-carvone, *α*-terpineol, (-)-terpinen-4-ol, (+)-pinocamphone, and (+)-isopinocamphone.

Each compound was prepared at three nominal release rates: low, medium, and high, corresponding to approximately 0.1, 1, and 10 mg day^{-1}, respectively. These release rates were selected to represent a range extending from levels close to natural host-tree emissions to higher rates potentially relevant to bark beetle management. Actual release rates were determined gravimetrically under both laboratory and field conditions. Laboratory measurements were conducted in a fume hood at 25 °C and an airflow velocity of 0.5 m s^{-1}. Field release rates were measured under environmental conditions corresponding to those experienced during the trapping experiments.

- Field experiments

Following dispenser calibration, field experiments were conducted at two forest sites in the Czech Republic. The first site was a 40-year-old Norway spruce stand near Libavá in North Moravia that had been affected by bark beetle outbreaks. The experiment at this site was conducted from 18 May to 3 June 2022.

The second site was located near Kostelec nad Černými lesy in a 70 - 90-year-old Norway spruce forest. Traps were installed in a two-year-old forest clearing, and the experiment was conducted from 3 June to 28 July 2022 (Figure 10A, B). The cross-vane panel traps were mounted on poles approximately 1.5 m above the ground. The traps were arranged in rows more than 30 m from the forest edge, with a minimum

spacing of 15 m between adjacent traps. Each trap was baited with a standard synthetic aggregation pheromone blend for *I. typographus*, consisting of 2-methyl-3-buten-2-ol and (*S*)-*cis*-Verbenol at a ratio of 10:1. Treatment traps were additionally baited with one of the eight synthetic host-associated compounds at a low, medium, or high release rate. Control traps contained only the aggregation pheromone. A Latin-square experimental design was used to minimize potential bias associated with trap position.

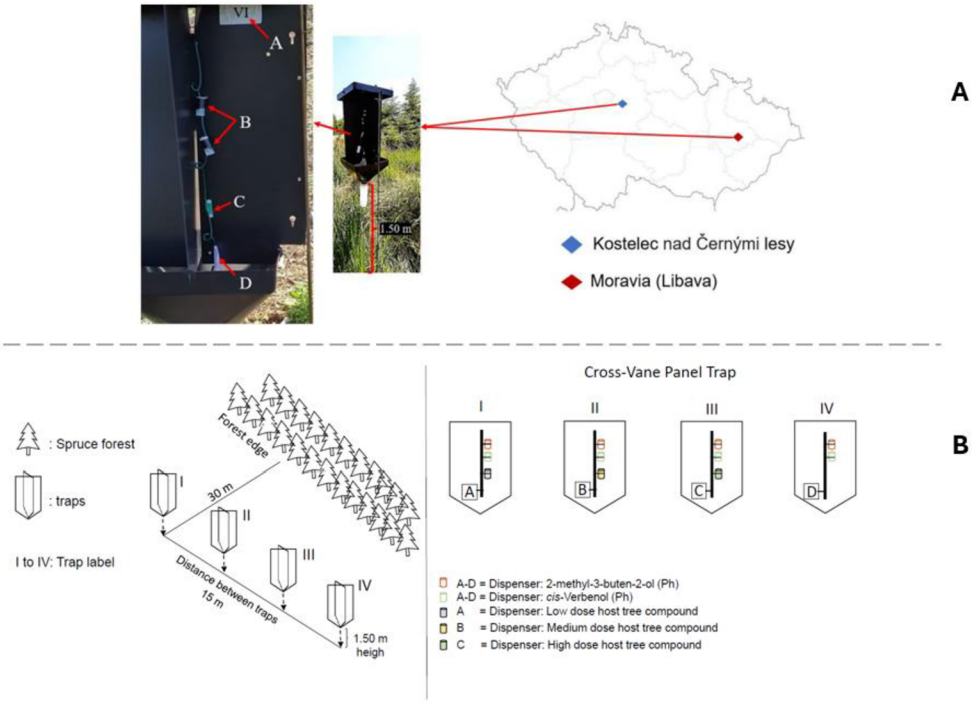

Figure 10. Locations and design of the field experiments conducted in the Czech Republic. A: Locations of the two study sites: Kostelec nad Černými lesy, indicated by a blue diamond, and Libavá, indicated by a red diamond. The trap and bait arrangement comprised: (A) a trap-identification label, (B) synthetic aggregation-pheromone dispensers, (C) a synthetic host-tree odour dispenser, and (D) a treatment-identification label. B: Experimental layout and trap arrangement. The left panel shows the spacing of traps I–IV in relation to the forest edge. The right panel presents a schematic cross-vane panel trap baited either with pheromone alone as the control treatment (Ph) or with pheromone combined with a host-associated compound at a specified release rate.

For each tested compound, one experimental block consisted of four traps: three treatment traps containing the compound at low, medium, or high release rates in combination with the aggregation pheromone, and one control trap containing the pheromone alone. Trap positions were rotated four times, and the experiment was

repeated twice, resulting in eight collection periods for each treatment. The captured insects were preserved in ethanol. Adult *I. typographus* were counted, whereas natural enemies were sorted and identified primarily to the family or genus level. These included pteromalid parasitoid wasps and staphylinid rove beetles, among other predatory and parasitoid taxa.

- Statistical analysis

The statistical analyses were based on the relative number of insects captured in each trap within each experimental block. Separate mixed-effects regression models were fitted for each tested compound. Trap identity nested within locality was included as a random effect to account for the hierarchical structure and repeated measurements of the experimental design.

4.3. From Field to Laboratory Experiments: Publication III

III. Moliterno, A. A. C., Shewale, M. K., Basile, S., Synek, J., & Jirošová, A. (2025). Size-dependent behavioural and antennal responses to doses of (+)-isopinocamphone and 1,8-cineole mixed with pheromone: A potential host-selection strategy in female *Ips typographus* L. Annals of Forest Science, 82(1), 34. https://doi.org/10.1186/s13595-025-01297-4

- Field experiments

Field-trapping experiments were conducted in 2019 and 2022 on forest property managed by the Czech University of Life Sciences Prague near Kostelec nad Černými lesy, Czech Republic. The study site was located in a mature Norway spruce forest. Traps were installed in a two-year-old clearing approximately 30 m from the forest edge, spaced at least 15 m apart, and mounted approximately 1.5 m above the ground. In both years, the experiments were conducted from 3 June to 28 July. The field experiments evaluated the behavioural responses of *Ips typographus* to three release

rates - low, medium, and high - of either 1,8-cineole or (+)-isopinocamphone. Each host-associated compound was presented in combination with the synthetic aggregation pheromone of *I. typographus*, whereas traps baited with the pheromone alone served as controls.

In 2019, seven cross-vane Ecotraps were used, and bait positions were rotated seven times according to a Latin-square design to minimize positional bias. In 2022, each compound was evaluated in experimental blocks consisting of four traps: three traps containing the compound at low, medium, or high release rates in combination with the pheromone, and one pheromone-only control. Trap positions were rotated four times, and the experiment was repeated twice, resulting in eight collection periods per treatment. The 2022 field layout followed the experimental design described for *Publication II*. The beetles collected from the field traps were preserved in ethanol. The field data were subsequently used to compare the sex ratio and female body size among the different odour treatments.

- Body-size and antennal morphometric analyses

For the body-size analysis, 50 beetles were randomly selected from each treatment group. The specimens were dried, sexed, and measured under a stereomicroscope, and damaged individuals were excluded from the analysis. The female body length was measured to determine whether attraction to the tested odour treatments differed according to body size. Antennal clubs were removed, mounted, photographed, and measured for length and width. These measurements were used to assess whether larger females possessed proportionally larger antennal sensory structures.

For the detailed morphometric analysis, total body length and antennal club length and width were measured in 30 large females and 30 small females. Standardized major axis regression was used to evaluate the scaling relationships between female body size and antennal dimensions while accounting for measurement error in both variables. A standardized major axis slope equal to 1 indicated an isometric

relationship, meaning that antennal dimensions increased in direct proportion to body size. Slopes greater than 1 indicated positive allometry, whereas slopes below 1 indicated negative allometry.

- Laboratory rearing and female size classification

For the laboratory experiments, *I. typographus* adults were obtained from naturally infested Norway spruce logs collected near Kostelec nad Černými lesy in 2024. The logs were maintained in fine-mesh emergence cages under controlled conditions and inspected daily. Newly emerged adult beetles were collected as they emerged. Only young females, approximately three days after emergence, were used in the antennal morphometric and electrophysiological experiments. Females were classified according to body length as large when they measured at least 4.80 mm and as small when they measured no more than 4.70 mm.

- Electroantennographic analysis

Electroantennography was used to evaluate whether antennal sensitivity differed between large and small female *I. typographus*. Antennae from both size classes were exposed to increasing doses of three odour treatments: the synthetic aggregation pheromone blend, 1,8-cineole, and (+)-isopinocamphone. The tested doses ranged from 0.001 to 1,000 µg. Each stimulus was applied to filter paper placed inside a glass Pasteur pipette and delivered to the antennal preparation as a 0.5-s pulse within a continuous stream of humidified air. Antennal responses were recorded as electrical depolarization amplitudes. This experimental design allowed the antennal sensitivity of large and small females to be compared across compounds and doses and helped determine whether female body size was associated with differences in olfactory sensitivity.

- Statistical analysis

Generalized linear models were used to test for differences in female body length among field treatments. Differences in sex ratio were evaluated using chi-square tests. Independent-samples t-tests were used to compare body size and antennal dimensions between large and small females. Standardized major axis regression was used to determine whether antennal club length and width scaled isometrically or allometrically with female body length. Electroantennographic responses of large and small females were compared at each odour dose using t-tests. All statistical analyses were performed in R version 4.1.1.

5. Results

This dissertation is based on the three published studies described in the Materials and Methods section. Publication I investigated the three-dimensional spatial distribution of *α*-pinene emitted by recently infested Norway spruce trees.

5.1. Main results: Publication I

I. Stříbrská, B., Moliterno, A. A. C., Hüttnerová, T., Leiner, M., Surový, P., & Jirošová, A. (2024). Pilot study of the 3D spatial distribution of α-pinene emitted by Norway spruce, *Picea abies* (L.) Karst., recently infested by *Ips typographus* (L. 1758) (Coleoptera: Scolytinae). Forests, 15(1), 10. https://doi.org/10.3390/f15010010

The first field trial was conducted at an air temperature of 25.1 °C, a relative humidity of 72.6%, and a wind speed of approximately 5 km h^{-1} from the southeast. Measurements obtained using solid-phase microextraction (SPME) fibers showed that the relative abundance of *α*-pinene was significantly higher at 5 cm from the stem of the infested tree than at 100 cm (Figure 11A). However, α-pinene abundance at 5 cm did not differ significantly from that recorded at 900 cm.

In contrast, samples collected using HayeSep-Q® sorbent cartridges showed no significant differences in α-pinene abundance among sampling distances, heights, or cardinal directions during the first field trial. The second field trial showed a broadly similar spatial pattern. For both SPME fibers and sorbent cartridges, α-pinene abundance differed significantly between sampling distances of 5 and 100 cm (Figure 11B). At 900 cm, which was considered the control zone, *α*-pinene abundance did not differ significantly from the values measured at the other distances.

No consistent significant effect of sampling height was detected in either field trial. Nevertheless, *α*-pinene abundance generally decreased between 130 and 400 cm above the ground for both collection methods, particularly during the first trial. Similarly, no consistent significant differences in *α*-pinene abundance were found among the four cardinal directions, regardless of the collection method or field trial (Figure 11A, B).

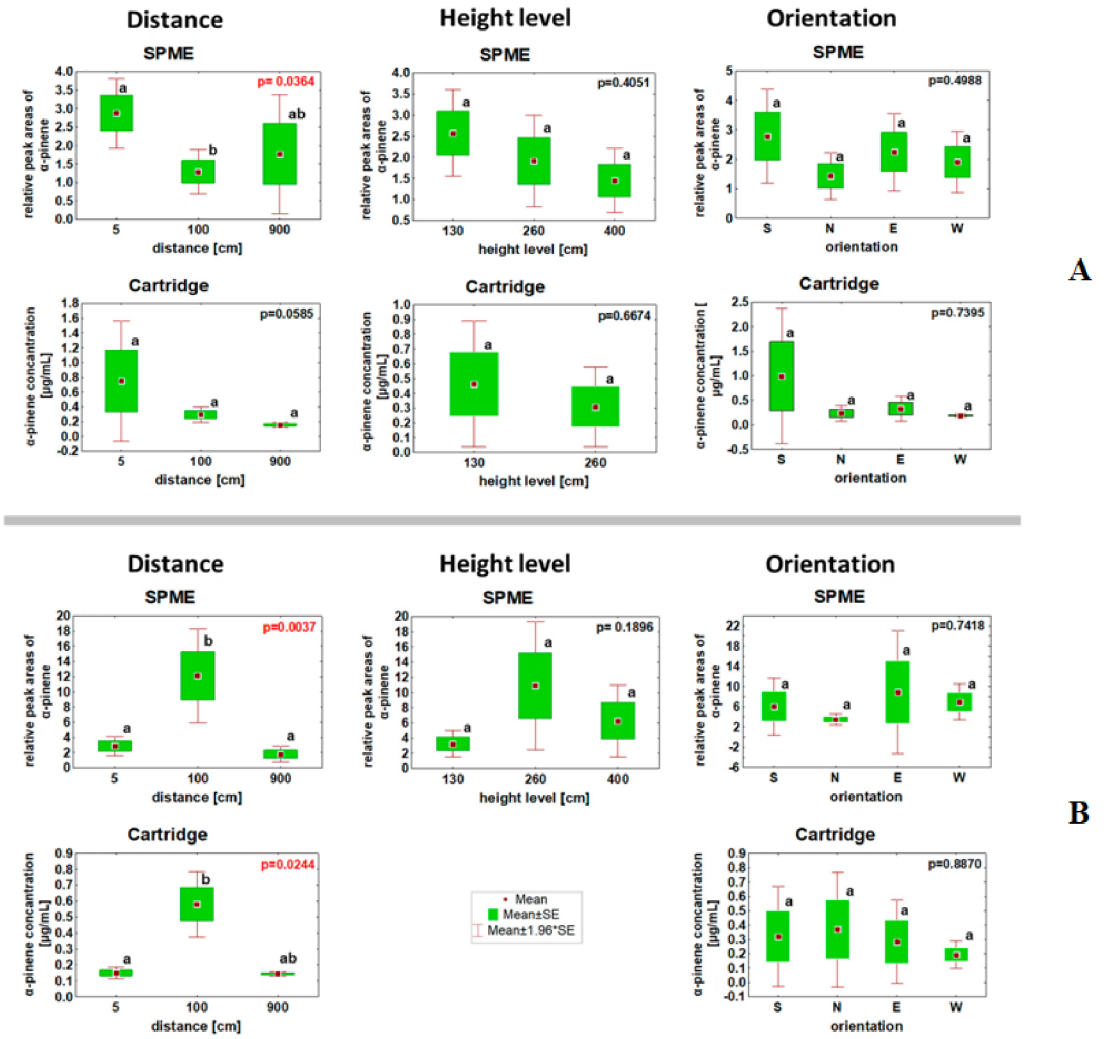

Figure 11. Spatial distribution of α-pinene around Norway spruce, *Picea abies*, trees recently infested by *Ips typographus* during the first (A) and second (B) field trials, conducted on 30 June and 24 August 2022, respectively. Results are presented, from left to right, according to sampling distance, height, and cardinal direction. The upper panels show samples collected using solid-phase microextraction fibers, whereas the lower panels show samples collected using HayeSep-Q® sorbent cartridges. Sampling distances were 5, 100, and 900 cm from the tree stem, and sampling heights were 130, 260, and 400 cm above the ground. Cardinal directions are indicated as S, south; N, north; E, east; and W, west. Small red squares represent means, green boxes represent means ± standard error, and whiskers represent means ± 1.96 × standard error. Different lowercase letters above the columns indicate significant differences among distances, heights, or cardinal directions. The reported p-values were obtained using Kruskal–Wallis tests. Sample sizes were N = 27 for SPME fibers and N = 12 for sorbent cartridges.

The three-dimensional distributions generated from the SPME measurements revealed substantial spatial variation in *α*-pinene abundance both among sampling positions and between the two field trials (Figure 12A, B).

During the first field trial, the highest *α*-pinene abundance, represented by the most saturated red coloration, was detected close to the tree stem at the lowest sampling height of 130 cm (Figure 12A). This pattern indicated that *α*-pinene accumulated most strongly near the lower part of the recently infested stem under the environmental conditions recorded during that trial. The second field trial showed higher and more variable α-pinene abundance than the first trial, with relative peak areas ranging from approximately 0.43 to 33.25 (Figure 12B). Although relatively high *α*-pinene abundance was again detected close to the stem at a distance of 5 cm, the measurements varied considerably among sampling heights and positions.

The spatial distribution also differed among cardinal directions and between field trials. During the first trial, the recorded wind speed was approximately 5 km h^{-1}, with wind originating from the southeast. During the second trial, the wind speed was approximately 3 km h^{-1} and originated from the south. These differences in wind conditions may have contributed to the observed variation in the direction and spatial extent of the *α*-pinene plume.

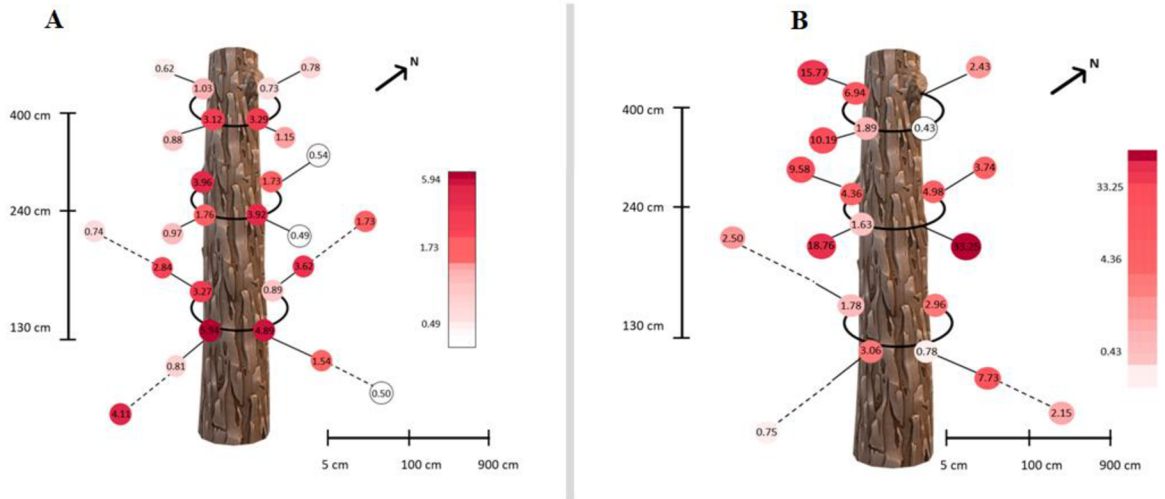

Figure 12. Three-dimensional distribution of α-pinene around Norway spruce, *Picea abies*, trees recently infested by *Ips typographus* during the first (A) and second (B) field trials. Sampling was conducted on 30 June 2022 during the first trial and on 24 August 2022 during the second trial. More saturated colors in the heat maps indicate a higher relative abundance of *α*-pinene. Numbers within the circles represent the relative peak areas of α-pinene. The horizontal and vertical axes indicate the distances and heights used to map the spatial distribution of *α*-pinene.

5.2. Main Results: Publication II

II. Moliterno, A. A. C., Jakuš, R., Modlinger, R., Unelius, C. R., Schlyter, F., & Jirošová, A. (2023). Field effects of oxygenated monoterpenes and estragole combined with pheromone on the attraction of *Ips typographus* and its natural enemies. Frontiers in Forests and Global Change, 6, 1292581. https://doi.org/10.3389/ffgc.2023.1292581

The results showed that oxygenated monoterpenes and estragole, when combined with the standard aggregation pheromone of *Ips typographus*, elicited compound- and dose-dependent behavioural responses compared with the pheromone-only control (Figure 13). The estragole significantly reduced the attraction of *I. typographus* at the high release rate, indicating an anti-attractant effect under these conditions (Figure 13A). Similarly, 1,8-cineole significantly reduced beetle attraction at all three release rates tested (Figure 13B). In contrast, (+)-isopinocamphone increased the attraction of *I. typographus* when combined with the aggregation pheromone, indicating a synergistic or attraction-enhancing effect (Figure 13C).

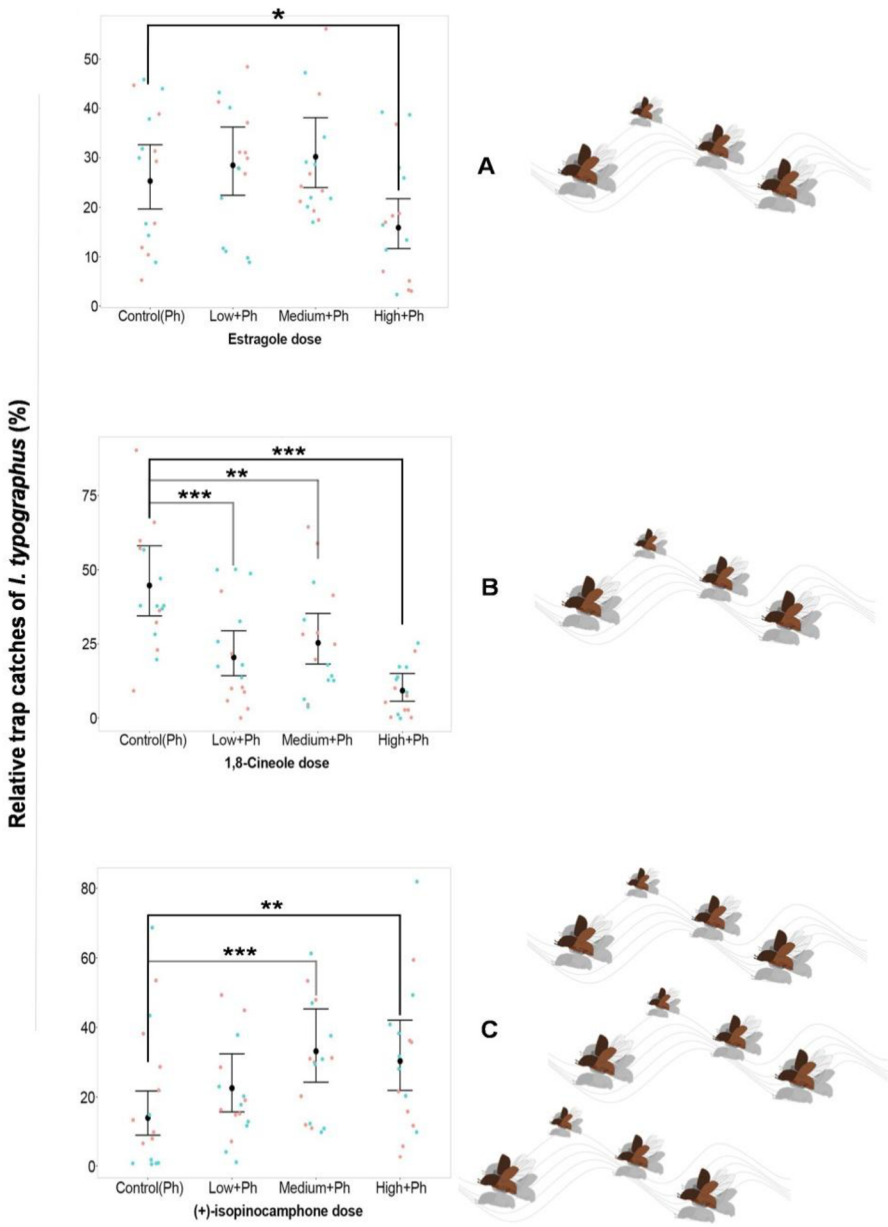

Figure 13. Relative numbers of *Ips typographus* captured in traps baited with the aggregation pheromone combined with low, medium, or high release rates of (A) estragole, (B) 1,8-cineole, or (C) (+)-isopinocamphone. Traps baited with the aggregation pheromone alone served as controls. Large black dots indicate posterior mean estimates, and vertical whiskers represent the corresponding 95% confidence intervals. Smaller dots represent the observed values from the individual field sites: blue, Kostelec nad Černými lesy; red, Moravia. Each compound and release-rate treatment was compared with the pheromone-only control using t-test contrasts. Asterisks indicate statistically significant differences from the control: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). Bark beetle image used as reference of bait-trap results evidenced. Image of the bark beetle adapted from Netherer et al. (2021).

None of the other tested compounds: (±)-camphor, (-)-carvone, *α*-terpineol, or (-)-terpinen-4-ol, produced a statistically significant difference in *I. typographus* attraction at any of the tested release rates when combined with the aggregation pheromone. The tested compounds also affected the catches of natural enemies associated with bark beetles. The two most abundant groups were pteromalid parasitoid wasps (Pteromalidae) and staphylinid rove beetles (Staphylinidae) (Figure 14).

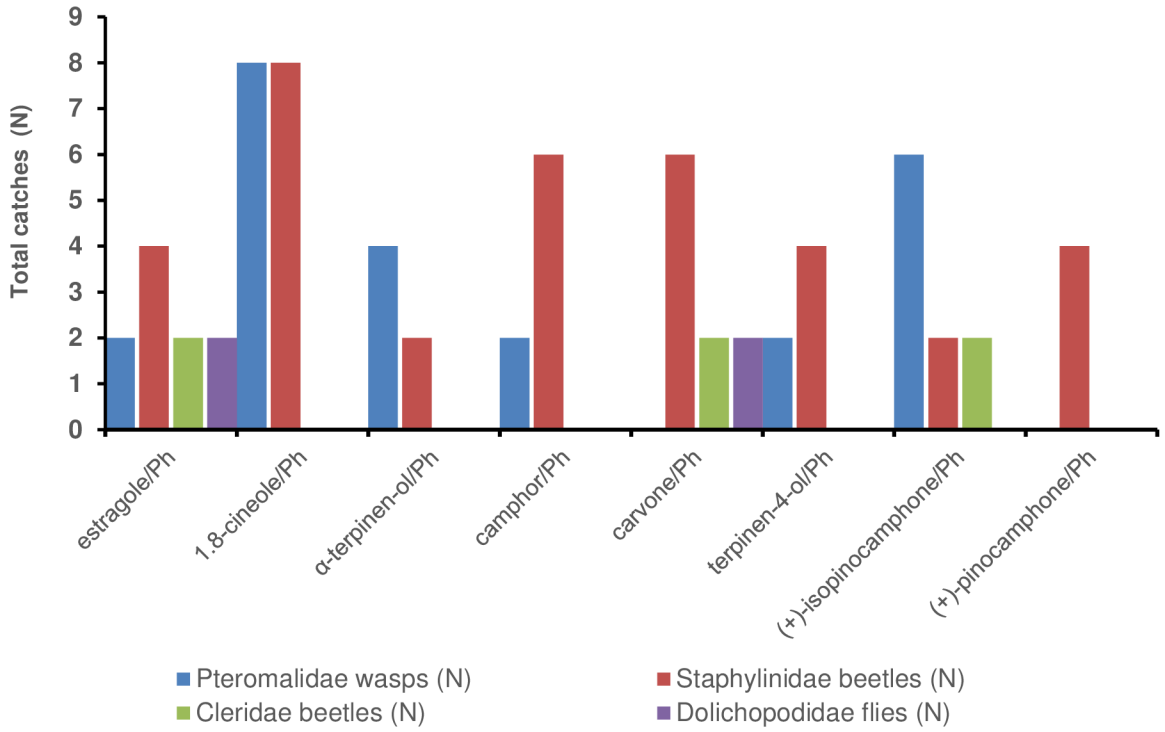

Figure 14. Total catches of the most abundant natural enemies of bark beetles: Pteromalid parasitoid wasps *Rhopalicus* sp. (Pteromalidae). Predators: rove beetles genus (Staphylinidae) (for genus names see. appendix, publication II, table 3.); long-legged fly *Medetera* sp. (Dolichopodidae) and *Thanasimus* sp. (Cleridae). Data were pooled by tested compound across the Moravia and Kostelec nad Černými lesy field experiments. Empty spaces referring zero catches. Catches below two were not considered. Control traps were baited with the aggregation pheromone alone (data not shown).

The most frequently recorded parasitoid was *Rhopalicus* sp. Its abundance differed markedly between the two study sites, with 13 individuals captured in Moravia and 93 individuals captured in Kostelec nad Černými lesy. The rove beetle catches were more similar between sites, with 23 individuals recorded in Moravia and 19 in Kostelec nad Černými lesy. The most abundant rove beetle genus at both locations was *Nudobius*

sp., represented by 10 individuals in Moravia and 7 individuals in Kostelec nad Černými lesy.

5.3. Main Results: Publication III

III. Moliterno, A. A. C., Shewale, M. K., Basile, S., Synek, J., & Jirošová, A. (2025). Size-dependent behavioural and antennal responses to doses of (+)-isopinocamphone and 1,8-cineole mixed with pheromone: A potential host-selection strategy in female *Ips typographus* L. Annals of Forest Science, 82(1), 34. https://doi.org/10.1186/s13595-025-01297-4

The results showed that the behavioural effects of (+)-isopinocamphone and 1,8-cineole were not strictly dose dependent. Instead, when these compounds were combined with the aggregation pheromone, they produced sex- and size-specific responses in wild populations of *Ips typographus* in the Czech Republic (Figure 14). In the field experiments conducted in 2019 and 2022, pheromone-baited traps supplemented with a high release rate of (+)-isopinocamphone captured larger females than traps containing the low release rate of this compound (Figure 15A). In contrast, traps baited with a high release rate of 1,8-cineole tended to capture smaller females, although the strength and consistency of this pattern differed between the two study years (Figure 15B).

Electroantennographic responses to the aggregation pheromone blend, consisting of 2-methyl-3-buten-2-ol and (*S*)-*cis*-Verbenol at a ratio of 10:1, did not differ significantly between large ($n = 10$) and small ($n = 10$) females at any of the tested doses (t-tests, $p > 0.05$; Figure 15C). In contrast, female size significantly affected antennal responses to (+)-isopinocamphone. Large females exhibited stronger electroantennographic responses than small females at the four highest doses tested: 1 µg ($t = 2.45$, $df = 18$, $p = 0.012$), 10 µg ($t = 2.83$, $df = 18$, $p < 0.01$), 100 µg ($t = 2.10$, $df = 18$, $p = 0.025$), and 1,000 µg ($t = 1.96$, $df = 18$, $p = 0.033$) (Figure 15D).

The opposite pattern was observed for 1,8-cineole. Large females exhibited significantly weaker electroantennographic responses than small females at 1 µg ($t = -2.14$, $df = 18$, $p = 0.023$), 10 µg ($t = -2.15$, $df = 18$, $p = 0.023$), 100 µg ($t = -2.52$, $df = 18$, $p = 0.011$), and 1,000 µg ($t = -2.75$, $df = 18$, $p = 0.007$) (Figure 15E). These contrasting physiological responses were consistent with the size-related differences observed in the field-trapping experiments.

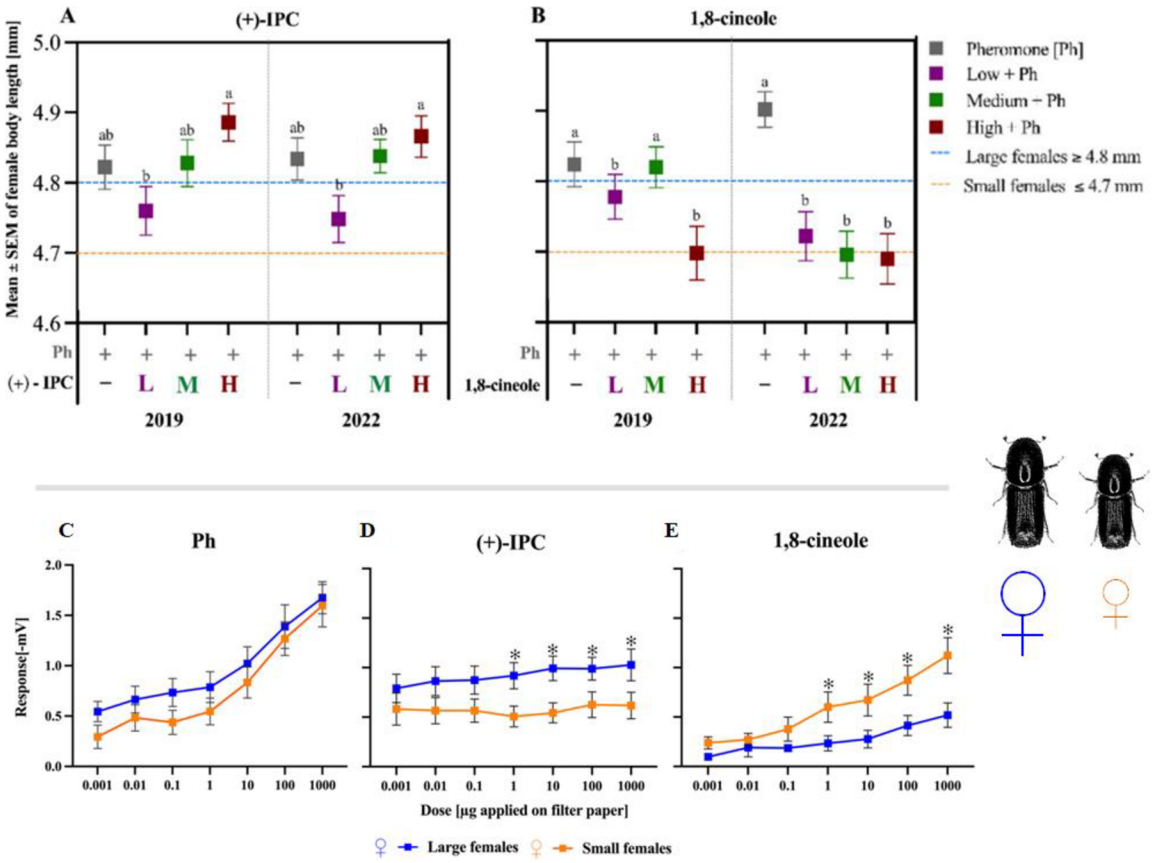

Figure 15. Body length and antennal responses of female *Ips typographus* belonging to different size classes. Mean body length ± standard error of females captured in pheromone-baited traps supplemented with low (L), medium (M), or high (H) release rates of (A) (+)-isopinocamphone [(+)-IPC] or (B) 1,8-cineole during the 2019 and 2022 field experiments. Traps baited with the aggregation pheromone alone (Ph) served as controls. Dashed horizontal lines indicate the thresholds used to classify females as large (≥ 4.80 mm) or small (≤ 4.70 mm). Different letters indicate significant differences among treatments within each year, based on Tukey's tests with Bonferroni adjustment ($p \leq 0.05$). Electroantennographic dose–response curves show the mean ± standard error responses of large and small females to (C) the aggregation pheromone, (D) (+)-isopinocamphone, and (E) 1,8-cineole across doses ranging from 0.001 to 1,000 µg. Responses were corrected against the hexane blank. Asterisks indicate significant differences between female size classes at individual doses (t-tests, $p < 0.05$). A representative image illustrating female body-size differences is shown on the right.

Body-size and antennal morphometric relationships, evidenced by morphometric analyses confirmed clear differences between the two female size classes. Body length differed significantly between small ($N = 30$) and large ($N = 30$) females ($t = 12.10$, $df = 58$, $p < 0.001$). Large females also had significantly longer antennal clubs ($t = 5.20$, $df = 58$, $p < 0.001$) and greater antennal club width ($t = 5.99$, $df = 58$, $p < 0.001$). A significant positive relationship was also detected between antennal club length and width in both size classes. This relationship explained 43% of the variation in large females ($R^2 = 0.43$, $p \leq 0.001$) and 32% of the variation in small females ($R^2 = 0.32$, $p = 0.001$). These findings indicate that larger females possess larger antennal clubs and that antennal dimensions increase in a coordinated manner within both female size classes.

6. Discussion

The three studies compiled in this dissertation expand current understanding of the chemical ecology of interactions between Norway spruce, *Picea abies*, and the European spruce bark beetle, *Ips typographus*. Together, they address three interconnected scales of chemical communication: the spatial distribution of α-pinene around recently infested trees, the effects of oxygenated monoterpenes and estragole on pheromone-mediated attraction, and phenotype-dependent behavioural and antennal responses in female *I. typographus*.

Considering these scales together is important because bark beetle infestation is not determined by a single factor or disturbance (Raffa 2001; Lehmanski et al., 2023). Successful host colonization arises from interactions among tree physiology and defense, volatile production and dispersal, beetle pheromone communication, microbial metabolism, natural enemies, environmental conditions, and the olfactory capacity of individual beetles (Netherer and Hammerbacher 2022; Ramakrishnan et al., 2025; Shewale et al., 2025; Six et al., 2026).

The findings of this dissertation therefore contribute to a more integrated understanding of both intraspecific and interspecific interactions within the spruce-bark beetle system. They show that spruce-associated volatiles may function as attractants, synergists, anti-attractants, or indicators of host condition. However, their ecological effects depend on several interacting factors, including the physiological condition of the host tree, compound concentration and release rate, spatial context, blend composition, environmental conditions, and beetle phenotype.

6.1. Spatial distribution of *α*-pinene around recently infested trees

Publication I demonstrated that *α*-pinene emitted by recently infested *P. abies* trees can be detected and spatially characterized under field conditions. This finding is

relevant to both the ecological interpretation of host volatile emissions and the development of methods for the early detection of bark beetle infestation.

α-Pinene is among the dominant monoterpenes emitted by Norway spruce, and its abundance may change following bark beetle attack or other forms of stress (Raffa 2014; Jaakkola et al., 2023). In particular, the host-derived enantiomer (-)-*α*-pinene is important because it can serve as a precursor in the biosynthesis of the aggregation pheromone component *cis*-Verbenol in *I. typographus* (Ramakrishnan et al., 2025). Consequently, the spatial distribution of *α*-pinene around attacked trees may provide information not only about host condition but also about the chemical environment in which pheromone communication occurs.

Following optimization of the volatile-collection procedure under laboratory conditions, the field measurements showed that *α*-pinene was not distributed uniformly around infested trees. During the first field trial, greater α-pinene abundance was generally detected close to the stem and at the lowest sampling height. In the second trial, however, the distribution differed, with relatively high abundance also detected farther from the stem and at intermediate heights.

The differences between the two trials emphasize that volatile plumes around attacked trees are dynamic rather than fixed. Their spatial structure may be affected by temperature, relative humidity, solar radiation, wind speed and direction, canopy structure, surrounding vegetation, and the physicochemical properties of the emitted compounds (Mezei et al., 2019; Stříbrská et al., 2022, 2023). The stage and intensity of infestation may also influence the amount and composition of volatiles released by the tree.

The principal contribution of Publication I is therefore not only the demonstration that chemical detection of recent bark beetle attack is feasible, but also the recognition that such detection is environmentally complex. Recently attacked trees may produce a

detectable chemical plume, but the location, concentration, and direction of that plume vary according to local abiotic and biotic conditions.

For this reason, volatile-based detection should not rely on measurements taken at a single fixed position. A more effective approach may combine spatially distributed chemical measurements with complementary detection technologies, such as electronic sensors, portable gas chromatography-mass spectrometry systems, electroantennographic detectors, remote sensing, and sensors mounted on unmanned aerial vehicles (Hüttnerová et al., 2023; Hüttnerová and Surový 2024; Aziz et al., 2025; Fang et al., 2026). Such integration could provide a dynamic representation of the volatile landscape and support the early identification of infested trees within integrated pest-management programs.

Monitoring compounds such as *α*-pinene may ultimately contribute to the development of chemical "fingerprints" associated with conifer stress or bark beetle attack. Nevertheless, *α*-pinene alone is unlikely to provide a fully specific indicator of infestation because it is also emitted by healthy, mechanically damaged, and otherwise stressed trees. Detection systems will therefore probably need to consider blends of compounds, changes in enantiomeric composition, and temporal emission patterns rather than the abundance of a single volatile.

6.2. Host-associated compounds as modifiers of pheromone-mediated attraction

Publication II showed that oxygenated monoterpenes and estragole are not merely components of the background odour of the host tree. Instead, some of these compounds act as biologically active signals that modify the pheromone-mediated behavior of *I. typographus*.

Among the tested compounds, 1,8-cineole produced the clearest inhibitory effect. When combined with the synthetic aggregation pheromone, it reduced beetle attraction at all tested release rates. This result is consistent with previous evidence that 1,8-

cineole interferes with attraction to the *I. typographus* aggregation pheromone and may indicate unsuitable, defended, or otherwise less favorable host material (Andersson et al., 2010; Jirošová et al., 2022; Schiebe et al., 2019; Netherer et al., 2021).

The physiological organization of the olfactory system provides further support for the behavioural importance of this compound. Olfactory sensory neurons responding to *cis*-Verbenol and 1,8-cineole occur in closely associated sensory structures in *I. typographus* (Andersson et al., 2010; Binyameen et al., 2014). Such peripheral organization may allow the beetle to evaluate an attractive pheromone signal together with a potentially inhibitory host-derived cue. The behavioural response would therefore reflect the balance between aggregation signals and information about host suitability.

Publication III extended this interpretation by showing that responses to 1,8-cineole also varied with female body size. Traps containing a high release rate of 1,8-cineole captured females with a smaller mean body size, although this pattern differed between years. In the laboratory, small females exhibited stronger electroantennographic responses to several doses of 1,8-cineole than large females. These findings suggest that female phenotype influences both the peripheral detection and behavioural evaluation of 1,8-cineole. However, antennal sensitivity alone cannot fully explain field behavior. Behavioural decisions also depend on central processing, integration with pheromone and host signals, previous experience, physiological condition, and the ecological costs and benefits of approaching a particular host.

Body-size variation in bark beetles may arise from larval competition, host nutritional quality, tree defenses, population density, and developmental conditions (Grodzki 2004; Sallé et al., 2005; Sallé and Raffa 2007). One possible hypothesis is that large and small females use host-related signals differently because they differ in competitive ability, energetic reserves, reproductive potential, or tolerance of host defenses. For example, smaller females may respond differently to chemical environments associated with lower-quality or more strongly defended hosts. This explanation remains hypothetical and requires direct testing.

Estragole also reduced the attraction of *I. typographus*, but only at the high release rate. The lack of inhibition at lower release rates indicates that its ecological function is concentration dependent. Estragole may therefore act as an anti-attractant only when its abundance exceeds a threshold or when its proportion within the total odour blend indicates an unsuitable host condition. Responses to estragole vary among bark beetle species. It has been reported to reduce trap catches of *Dendroctonus brevicomis* LeConte, *D. simplex*, and *D. rufipennis*, whereas increased catches have been observed for *D. frontalis* and *D. terebrans* under some conditions (Hayes and Strom, 1994; Munro et al., 2020). Reduced catches have also been reported in Nearctic *Ips* species, including *Ips pini* and *I. latidens* (Joseph et al., 2001).

These contrasting responses may reflect differences in host preference, evolutionary history, olfactory specialization, compound concentration, and the ecological context in which estragole is encountered. They further demonstrate that the behavioural function of a volatile cannot necessarily be generalized across bark beetle taxa. Beetles are likely to evaluate the balance among pheromones, host volatiles, microbial metabolites, visual information, and indicators of tree physiological condition rather than responding to individual compounds in isolation (Netherer et al., 2021; Kandasamy et al., 2023; Lehmanski et al., 2023).

6.3. (+)-Isopinocamphone as an attraction-enhancing signal

In contrast to the inhibitory effects of 1,8-cineole and high-release-rate estragole, (+)-isopinocamphone enhanced attraction to the aggregation pheromone, particularly at medium and high release rates. This finding indicates that some oxygenated monoterpenes can function as pheromone synergists rather than anti-attractants. One possible explanation is that (+)-isopinocamphone helps beetles identify stressed, colonized, or otherwise suitable host material. The compound has also been associated with ophiostomatoid fungi linked to *I. typographus*, suggesting that it may contribute to interactions among the host tree, fungal symbionts, and bark beetles (Kandasamy et

al., 2023). In this context, the compound could indicate that microbial activity has altered the chemical environment of the host or that colonization is already in progress. Publication III provided evidence that the response to (+)-isopinocamphone also depends on female phenotype. Field traps baited with the compound and pheromone captured larger females under specific release-rate conditions. Large females also possessed larger antennal clubs and exhibited stronger electroantennographic responses to the four highest tested doses of (+)-isopinocamphone.

The agreement between the field and laboratory findings suggests that larger females may have a greater capacity to detect or respond to this compound. Larger antennal clubs may contain a greater number of olfactory sensilla or provide a larger sensory surface, although sensillum abundance and distribution were not measured directly in the present research.

From an ecological perspective, size-dependent host selection may be especially important in female *I. typographus*, because females initiate gallery construction and oviposition. Their selection of a host or breeding site therefore directly influences reproductive performance and offspring survival (Pureswaran and Borden 2003; Foelker and Hofstetter 2014). If larger females detect (+)-isopinocamphone more effectively, they may gain an advantage when locating hosts that are stressed, fungus-colonized, or otherwise favorable for reproduction. Nevertheless, a stronger antennal response should not automatically be interpreted as better host-selection ability. Additional studies are required to determine whether the observed physiological differences lead to greater reproductive success, improved colonization, or more efficient use of host resources.

6.4. Compounds without significant field effects

Camphor, carvone, *α*-terpineol, and terpinen-4-ol did not significantly affect trap catches when presented individually with the aggregation pheromone at the tested release rates. This absence of a measurable field response is informative because

these compounds have previously been shown to elicit antennal activity in *I. typographus* (Kalinová et al., 2014; Schiebe et al., 2019). The difference between antennal detection and field behavior demonstrates that electrophysiological activity alone is insufficient to predict ecological function. A compound may be detected by the antenna without producing attraction or inhibition under field conditions. Its effect may depend on concentration, enantiomeric composition, interactions with other compounds, environmental conditions, or the physiological state of the responding beetle.

For example, racemic (±)-camphor was used in the field experiments, whereas the separate enantiomers may differ in biological activity. Evaluating the effects of individual enantiomers and naturally occurring enantiomeric ratios would help clarify the specificity of olfactory responses and their role in host-seeking behavior in *Ips* species (Blažytė-Čereškienė et al., 2016; Jirošová et al., 2022; Shewale et al., 2026). The inactive compounds should also be tested as components of more complex blends. They may not affect behavior individually but could modify responses when combined with one another, with microbial volatiles, or with compounds such as 1,8-cineole and (+)-isopinocamphone.

6.5. Responses of natural enemies

The responses of bark beetle natural enemies were less conclusive than those of *I. typographus*. Pteromalid parasitoid wasps and staphylinid rove beetles were captured in the field traps, but their abundance differed between localities, and no strong or consistent preference for a specific host-associated compound was detected.

This variation may reflect differences in bark beetle abundance, natural-enemy population size, forest structure, season, weather, prey availability, and the suitability of the traps for capturing parasitoids and predators (Welch and Harwood 2014; Wegensteiner et al., 2015). Trap design may have been particularly important because small parasitoid wasps may escape from traps designed primarily for bark beetles, leading to an underestimation of their abundance.

Even without clear treatment-specific responses, the capture of predators and parasitoids in pheromone-baited traps confirms that semiochemical manipulation may affect organisms beyond the target pest. The development of operational lures or anti-attractants should therefore consider possible effects on associated trophic interactions. Management methods that increase pest catches but also disrupt natural enemies may have unintended ecological consequences.

6.6. Research limitations and recommendations for future studies

Despite the contributions of these studies, several limitations should be recognized. In *Publication I* was a pilot investigation based on a limited number of infested trees and field trials. Broader replication is needed across forest stands, seasons, weather conditions, stages of infestation, tree health categories, and infestation densities. Measurements around uninfested control trees and trees affected by non-insect stress would also help determine how specifically the observed volatile patterns indicate bark beetle attack.

Future studies should improve the temporal and spatial resolution of VOC sampling. Portable gas chromatography-mass spectrometry systems, sensor arrays, automated sampling devices, and sensors mounted on unmanned aerial vehicles could be evaluated alongside conventional sorbent and SPME methods. Conducting comparable trials across different regions and neighboring countries would help determine whether the observed patterns are transferable across forest types and climatic conditions.

A further limitation of *Publication I* was that *α*-pinene was not resolved into its individual enantiomers. This distinction may be biologically important because the pheromone component *cis*-Verbenol is associated particularly with metabolism of host-derived (-)-*α*-pinene rather than (+)-*α*-pinene (Ramakrishnan et al., 2025). Future analyses should

therefore quantify both *α*-pinene enantiomers and evaluate changes in their ratios during the progression of infestation.

Publication II demonstrated biologically meaningful effects of selected compounds, but further research is required to determine optimal release rates, effective deployment periods, blend interactions, trap spacing, and possible effects on non-target organisms. The effects of *α*-terpineol, camphor, carvone, and terpinen-4-ol should also be evaluated in binary or ternary blends because combinations may produce responses that were not detected when the compounds were tested individually. In addition, the field-trap design represented another limitation. The traps were developed primarily to capture bark beetles and may not efficiently retain small parasitoids or highly mobile predators. Alternative traps, retention systems, direct observations, and automated monitoring could provide more accurate estimates of natural-enemy responses.

One treatment involving the medium release rate of pinocamphone at the Kostelec site was also affected by unintended opening of the dispenser, probably because of a defective lid combined with warm weather. This malfunction may have altered the actual release rate and should be considered when interpreting that treatment. Future experiments should include more robust dispenser materials, repeated gravimetric checks, and field-based monitoring of release rates throughout the experimental period.

Publication III provided evidence of size-dependent behavioural and antennal responses in females, but the underlying mechanisms remain uncertain. Comparable analyses should be extended to pioneer males, additional female reproductive stages, outbreak and non-outbreak populations, and other ecologically important semiochemicals. (+)-*trans*-4-thujanol would be especially relevant because of its importance in the chemical communication of female *I. typographus* (Jirošová et al., 2022). Future studies should also quantify antennal sensilla to determine whether larger antennal clubs contain more receptor structures or differ in sensillum type and distribution. Behavioural assays under controlled laboratory conditions could help

distinguish attraction, avoidance, and arrestment responses and could be combined with electrophysiology, transcriptomic analyses, and receptor-level studies.

Most importantly, future research should integrate spatial VOC mapping, behavioural trapping, and physiological measurements within the same experimental framework. Such an approach would directly connect the volatile plume surrounding a tree, the chemical composition of the host and lure, the environmental context, and the phenotype of the responding beetles.

6.7. Practical applications of the research findings

The findings of this dissertation have several implications for the monitoring and management of *I. typographus*. First, spatial measurements of α-pinene show that host volatiles may provide detectable information about recent attack and tree condition. Although *α*-pinene alone is unlikely to serve as a specific diagnostic marker, it could form part of a multicomponent chemical-detection system for identifying potentially infested trees.

Second, 1,8-cineole and high-release-rate estragole reduced pheromone-mediated attraction and may therefore be candidates for the “push” component of Push-Pull management strategies. These compounds could potentially be deployed around vulnerable trees, forest edges, timber-storage areas, or other protected zones to reduce beetle approach. However, their effectiveness must be confirmed at operational scales and under a wider range of environmental conditions.

Third, (+)-isopinocamphone enhanced pheromone-mediated attraction and may be useful in the “pull” component of such strategies. Incorporating this compound into pheromone-baited traps could potentially increase trap sensitivity or improve the detection of dispersing beetles. Its practical value will depend on determining an effective and stable release rate and confirming that enhanced attraction produces meaningful improvements in monitoring or mass trapping.

The observed effects on natural enemies emphasize that semiochemical-based management should be evaluated at the community level rather than solely in terms of pest catches. Ideally, management compounds should strongly affect *I. typographus* while producing minimal disruption to predators, parasitoids, and other non-target organisms.

Finally, the results of *Publication III* show that *I. typographus* populations cannot be assumed to respond uniformly to chemical signals. Female body size and antennal morphology were associated with differences in behavioural and physiological responses. Variation among individuals may therefore influence the effectiveness of lures, repellents, and other semiochemical tools.

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7. Conclusion

The three studies presented in this dissertation show that volatile organic compounds and semiochemicals are not only signs of bark beetle infestation. They actively influence how *Ips typographus* locates, evaluates, and selects Norway spruce trees. Together, the results suggest that host selection occurs through three connected steps.

First, a stressed or infested tree releases a volatile plume containing major compounds such as *α*-pinene and smaller amounts of biologically active compounds. The position and strength of this plume change with distance, height, wind, and other environmental conditions. Second, beetles detect these host volatiles together with aggregation pheromones. Their response depends on the identity and concentration of each compound. In this dissertation, 1,8-cineole and high doses of estragole reduced pheromone-mediated attraction, whereas (+)-isopinocamphone increased attraction. Third, beetles do not all respond in the same way. Female body size, antennal morphology, and antennal sensitivity influenced how some compounds were detected and how females responded to them. Larger and smaller females therefore appeared to process certain host-related signals differently.

These findings help explain why bark beetle responses to semiochemicals can vary among individuals, years, study sites, and environmental conditions. They also show that a compound cannot always be classified simply as attractive or repellent. Its effect depends on concentration, blend composition, host condition, environmental context, and the characteristics of the responding beetle. The main contribution of this dissertation is the demonstration that host selection in *I. typographus* is a dynamic process shaped by the interaction between the tree, the surrounding chemical environment, and the sensory characteristics of the beetle. By combining spatial volatile measurements, field trapping, body-size analysis, antennal morphology, and electrophysiology, this research links processes that are often studied separately.

The results also provide a basis for developing more sustainable forest-protection methods. *α*-Pinene and other host volatiles may contribute to early infestation

detection. Compounds such as 1,8-cineole and estragole may be useful as anti-attractants in the “push” component of pest-management strategies, whereas (+)-isopinocamphone may improve the “pull” component by increasing the effectiveness of pheromone-baited traps. However, these compounds must be tested further under different forest conditions, release rates, and population phases before they can be used operationally. Their effects on natural enemies and other non-target organisms must also be considered.

In conclusion, effective management of *I. typographus* requires more than identifying single attractive or repellent compounds. It requires an integrated understanding of how trees release chemical signals, how these signals spread through the forest, and how beetles with different phenotypes detect and respond to them. This perspective provides a strong foundation for improving early detection, refining semiochemical-based management, and reducing reliance on conventional insecticides under increasing bark beetle pressure associated with climate change.

6. References

Ait, K., Palm-Hellenurm, K., Õunap, H., Kaart, T., Vodde, F., Orumaa, A., &Metslaid, M. (2025). Trap log felling methods drive bark beetle communities without affecting *Ips typographus* (L.) densities on Norway spruce. European Journal of Forest Research, 144, 683–698. https://doi.org/10.1007/s10342-025-01787-0

Alamouti, M. S., Kim, J. J., Humble, L. M., Uzunovic, A., &Breuil, C. (2007). Ophiostomatoid fungi associated with the northern spruce engraver, Ips perturbatus, in western Canada. Antonie van Leeuwenhoek, 91(1), 19-34.https://doi.org/10.1007/s10482-006-9092-8

Austarå, Ø., & Midtgaard, F. (1986). On the longevity of *Ips typographus* L. adults. Journal. of Applied Entomology, 102(1-5), 106-111. https://doi.org/10.1111/j.1439-0418.1986.tb00898.x

Aukema, B. H., & Raffa, K. F. (2005). Selective manipulation of predators using pheromones: responses to frontalin and ipsdienol pheromone components of bark beetles in the Great Lakes region. Agricultural and Forest Entomology, 7(3), 193-200.https://doi.org/10.1111/j.1461-9555.2005.00250.x

Anderbrant, O., Schlyter, F., & Birgersson, G. (1985). Intraspecific competition affecting parents and offspring in the bark beetle Ips typographus. Oikos, 89-98.

Anderbrant, O. (1986). A model for the temperature and density dependent reemergence of the bark beetle *Ips typographus*. Entomologia Experimentalis et Applicata, 40, 81–88.

Anderbrant, O., & Schlyter, F. (1989). Causes and effects of individual quality in bark beetles. Holarctic Ecology, 12, 488-493.

Anderbrant, O. (1990). Gallery construction and oviposition of the bark beetle *Ips typographus* (Coleoptera: Scolytidae) at different breeding densities. Ecological Entomology, 15, 1-8.

Andersson, M. N., Larsson, M. C., & Schlyter, F. (2009). Specificity and redundancy in the olfactory system of the bark beetle Ips typographus: single-cell responses

to ecologically relevant odors. Journal of Insect Physiology, 55(6), 556-567. https://doi.org/10.1016/j.jinsphys.2009.01.018

Andersson, M. N., Larsson, M. C., Blaženec, M., Jakuš, R., Zhang, Q. H., & Schlyter, F. (2010). Peripheral modulation of pheromone response by inhibitory host compound in a beetle. Journal of Experimental Biology, 213(19), 3332-3339.https://doi.org/10.1242/jeb.044396

Anderson, R. S. (2002). Curculionidae Latreille 1802. In: Arnett, R. H., Thomas, M. C., Skelley, P. E., & Frank, J. H. (Eds.). American Beetles, Volume II: Polyphaga: Scarabaeoidea through Curculionoidea (Vol. 2). CRC press.

Andersson M.N. (2012). Mechanisms of odor coding in coniferous bark beetles: from neuron to behavior and application. Psyche A Journal ofEntomology.2012:1–14. https://doi.org/10.1155/2012/149572

Annila, E. (1969). Influence of temperature upon the development and voltinism of Ipstypographus L. (Coleoptera, Scolytidae). Annales Zoologici Fennici, 6, 161–208.

Aziz, D., Rafiq, S., Saini, P., Ahad, I., Gonal, B., Rehman, S. A., ... & Nabila Iliya, M. (2025). Remote sensing and artificial intelligence: revolutionizing pest management in agriculture. Frontiers in Sustainable Food Systems, 9, 1551460.https://doi.org/10.3389/fsufs.2025.1551460

Bai, Z., Fang, J., Yu, C., Zhang, S., Liu, F., Han, F., ... & Kong, X. (2024). Divergent response of two bark beetle–fungal symbiotic systems to host monoterpenes reflects niche partitioning strategies. Journal of Chemical Ecology, 50(12), 994-1005.https://doi.org/10.1007/s10886-024-01535-5

Baier, P., Pennerstorfer, J. & Schopf, A. (2007) PHENIPS4A comprehensive phenology model of *Ips typographus* (L.) (Col., Scolytinae) as a tool for hazard rating of bark beetle. https://doi.org/10.1016/j.foreco.2007.05.020

Bakke, A. (1973). Bark beetle pheromones and their potential use in forestry. EPPO Bulletin, 2(9), 5-15.https://doi.org/10.1111/j.1365-2338.1973.tb02096.x

Bakke, A. (1981). Inhibition of the response in *Ips typographus* to the aggregation pheromone; field evaluation of verbenone and ipsenol. Zeitschrift für

angewandteEntomologie, 92(1-5), 172-177. https://doi.org/10.1111/j.1439-0418.1981.tb01666.x

Basile, S., Stříbrská, B., Kalyniukova, A., Hradecký, J., Synek, J., Gershenzon, J., & Jirošová, A. (2024). Physiological and biochemical changes of *Picea abies* (L.) during acute drought stress and their correlation with susceptibility to *Ips typographus* (L.) and *I. duplicatus* (Sahlberg). Frontiers in Forests and Global Change, 7, 1436110.https://doi.org/10.3389/ffgc.2024.1436110

Beetz, K., Marrs, C., Busse, A., Poděbradská, M., Kinalczyk, D., Kranz, J., & Forkel, M. (2025). Effects of bark beetle disturbance and fuel types on fire radiative power and burn severity in the Bohemian-Saxon Switzerland. Forestry: An International Journal of Forest Research, 98(1), 59-70.https://doi.org/10.1093/forestry/cpae024

Bergström, G. (2007). Chemical ecology= chemistry+ ecology!. Pure and Applied Chemistry, 79(12), 2305-2323.https://doi.org/10.1351/pac200779122305

Berisford, C. W. (2011). Parasitoids of the southern pine beetle. In R. N. Coulson & K. D. Klepzig (Eds.), Southern pine beetle II (Gen. Tech. Rep. SRS-140). U.S. Department of Agriculture, Forest Service, Southern Research Station. https://research.fs.usda.gov/treesearch/39025

Biedermann, P. H. W., Müller, J., Grégoire, J.-C., Gruppe, A., Hagge, J., Hammerbacher, A., Hofstetter, R. W., Kandasamy, D., Kolarik, M., Kostovcik, M., Krokene, P., Sallé, A., Six, D. L., Turrini, T., Vanderpool, D., Wingfield, M. J., &Bässler, C. (2019). Bark beetle population dynamics in the Anthropocene: Challenges and solutions. Trends in Ecology & Evolution, 34(10), 914–924. https://doi.org/10.1016/j.tree.2019.06.002

Binyameen, M., Jankuvová, J., Blaženec, M., Jakuš, R., Song, L., Schlyter, F., & Andersson, M. N. (2014). Co-localization of insect olfactory sensory cells improves the discrimination of closely separated odour sources. Functional ecology, 28(5), 1216-1223.https://doi.org/10.1111/1365-2435.12252

Birgersson, G., Dalusky, M. J., & Berisford, C. W. (2000). Identification of an aggregation pheromone for Pityogeneshopkinsi (Coleoptera: Scolytidae).

TheCanadian Entomologist, 132(6), 951-963. https://doi.org/10.4039/Ent132951-6

Birch, M. C. (1984). Aggregation in bark beetles. In Bell, W. J., Cardé. R. T (1. Ed.)., Chemical ecology of insects (pp. 331-353). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4899-3368-3

Bonetti, F., Gabrielaitis, L., Bassanelli, S., Hamid, F., Cosio, L., & Marconi, A. (2026, March). Beetle Migration Game: Balancing Ecosystems as a More-than-Human Play Practice. In Proceedings of the 10th Annual International GamiFIN Conference 2026 (pp. 1-6).https://doi.org/10.1145/3789624.3789647

Boone, C. K., Six, D. L., Zheng, Y., & Raffa, K. F. (2014). Parasitoids and dipteran predators exploit volatiles from microbial symbionts to locate bark beetles. Environmental Entomology, 37(1), 150-161. https://doi.org/10.1093/ee/37.1.150

Bozzini, A., Missoni, M., Santoiemma, G., Bernardinelli, I., Comino, R., & Faccoli, M. (2026). Sanitation felling against the European spruce bark beetle: A matter of intensity and forest type. Agricultural and Forest Entomology, 1–16. https://doi.org/10.1111/afe.70039

Botterweg, P. F. (1982). Dispersal and flight behaviour of the spruce bark beetle *Ips typographus* in relation to sex, size and fat content. Zeitschrift für angewandteEntomologie, 94(1-5), 466-489.https://doi.org/10.1111/j.1439-0418.1982.tb02594.x

Bullor, L., Braude, H., Monzón, J., Cotes Prado, A. M., Casavola, V., Carbajal Morón., N. & Risopoulos, J. 2024. Bioinputs: Investment opportunities in Latin America. Directions in investment No. 9. Rome, FAO. https://doi.org/10.4060/cc9060en

Bullington, L., Martin, E., Lekberg, Y., Sadyrova, M., & Six, D. L. (2026). Bark beetle–fungi symbioses reshape nutrient dynamics, fungal decomposer succession, and decay rates in ponderosa pine. New Phytologist, 249(4), 2041-2054.https://doi.org/10.1111/nph.70778

Butenandt, V. A. (1959). Uber den sexsual-lockstoff des seidenspinners Bombyx mori. Reindarstellung und konstitution. Z. Naturforschg, b, 14, 283.

Blake, M., Straw, N., Kendall, T., Whitham, T., Manea, I. A., Inward, D., Jones, B., Hazlitt, N., Ockenden, A., Deol, A., Brown, A., Ransom, E., Smith, L., & Facey, S. (2024). Recent outbreaks of the spruce bark beetle *Ips typographus* in the UK: Discovery, management, and implications. Trees, Forests and People, 16, Article 100508.https://doi.org/10.1016/j.tfp.2024.100508

Blažytė-Čereškienė, L., Apšegaitė, V., Radžiutė, S., Mozūraitis, R., Būda, V., &Pečiulytė, D. (2016). Electrophysiological and behavioural responses of Ipstypographus (L.) to trans-4-thujanol—a host tree volatile compound. Annals of Forest Science, 73(2), 247-256.https://doi.org/10.1007/s13595-015-0494-5

Bleiker, K. P., & Six, D. L. (2014). Dietary benefits of fungal associates to an eruptive herbivore: potential implications of multiple associates on host population dynamics. Environmental Entomology, 36(6), 1384-1396. https://doi.org/10.1093/ee/36.6.1384

Blomquist, G. J., Figueroa-Teran, R., Aw, M., Song, M., Gorzalski, A., Abbott, N. L., ... & Tittiger, C. (2010). Pheromone production in bark beetles. Insect biochemistry and molecular biology, 40(10), 699-712.https://doi.org/10.1016/j.ibmb.2010.07.013

Bracalini, M., Martini, A., Tagliaferri, L., & Panzavolta, T. (2025). Impact of trapping programs for *Ips typographus* (Linnaeus) (Curculionidae: Scolytinae) on predators, parasitoids, and other non-target insects. Forests, 16, Article 1510. https://doi.org/10.3390/f16101510

Byers, J. A., Birgersson, G., Löfqvist, J., & Bergström, G. (1988). Synergistic pheromones and monoterpenes enable aggregation and host recognition by a bark beetle. Naturwissenschaften, 75(3), 153-155.

Byers, J. A. (1989). Behavioural mechanisms involved in reducing competition in bark beetles. Ecography, 12(4), 466-476.https://doi.org/10.1111/j.1600-0587.1989.tb00924.x

Byers, J. A., Birgersson, G., Löfqvist, J., Appelgren, M., & Bergström, G. (1990). Isolation of pheromone synergists of bark beetle, Pityogeneschalcographus, from complex insect-plant odors by fractionation and subtractive-combination

bioassay. Journal of chemical ecology, 16(3), 861-876.https://doi.org/10.1007/BF01016496

Byers, J. A., Zhang, Q. H., & Birgersson, G. (2000a). Strategies of a bark beetle, Pityogenesbidentatus, in an olfactory landscape. Naturwissenschaften, 87(11), 503-507.https://doi.org/10.1007/s001140050768

Byers, J. A., Zhang, Q. H., & Birgersson, G. (2000b). Strategies of a bark beetle, Pityogenesbidentatus, in an olfactory landscape. Naturwissenschaften, 87(11), 503-507.https://doi.org/10.1007/s00114-004-0520-1

Byers, J. A. (2007). Chemical ecology of bark beetles in a complex olfactory landscape. In F. Lieutier, K. R. Day, A. Battisti, J.-C. Grégoire, & H. F. Evans (Rep. Eds.), Bark and wood boring insects in living trees in Europe: A synthesis(pp. 89–134). Springer. https://doi.org/10.1007/978-1-4020-2241-8_5

Byers, J. A., Birgersson, G., & Francke, W. (2013). Aggregation pheromones of bark beetles, Pityogenesquadridens and P. bidentatus, colonizing Scotch pine: olfactory avoidance of interspecific mating and competition. Chemoecology, 23(4), 251-261.https://doi.org/10.1007/s00049-013-0139-9

Cale, J. A., Ding, R., Wang, F., Rajabzadeh, R., & Erbilgin, N. (2019). Ophiostomatoid fungi can emit the bark beetle pheromone verbenone and other semiochemicals in media amended with various pine chemicals and beetle-released compounds. Fungal Ecology, 39, 285-295.https://doi.org/10.1016/j.funeco.2019.01.003

Capra, F. (1996). The web of life: A new synthesis of mind and matter (Vol. 132). London: HarperCollins.

Carlson, L., Müller, J., Bevanda, M., Biedermann, P., Bradler, P. M., Dittrich, S., Lira Dyson, B., Fichtner, A., Castañeda-Gomez, A. J., Von Oheimb, G., Pierick, K., Pflumm, L., Mitesser, O., Rothacher, J., Wild, C., & Schmitt, T. (2025). The invisible heterogeneity of a forest – beta diversity of volatiles. https://doi.org/10.1101/2025.09.03.673352

Cook, S. M., Khan, Z. R., & Pickett, J. A. (2007). The use of push–pull strategies in integrated pest management. Annual Review of Entomology, 52, 375–400. https://doi.org/10.1146/annurev.ento.52.110405.091407

Cognato, A. I. (2015). Biology, systematics, and evolution of Ips. In Bark beetles (pp. 351-370). Academic Press.https://doi.org/10.1016/B978-0-12-417156-5.00009-5

Chakraborty, A., Modlinger, R., Ashraf, M. Z., Synek, J., Schlyter, F., & Roy, A. (2020a). Core mycobiome and their ecological relevance in the gut of five Ips bark beetles (Coleoptera: Curculionidae: Scolytinae). Frontiers in Microbiology, 11, 568853.https://doi.org/10.3389/fmicb.2020.568853

Chakraborty, A., Ashraf, M. Z., Modlinger, R., Synek, J., Schlyter, F., & Roy, A. (2020b). Unravelling the gut bacteriome of Ips (Coleoptera: Curculionidae: Scolytinae): identifying core bacterial assemblage and their ecological relevance. Scientific reports, 10(1), 18572.https://doi.org/10.1038/s41598-020-75203-5

Chakraborty, A., Purohit, A., Khara, A., Modlinger, R., & Roy, A. (2023). Life-stage and geographic location determine the microbial assemblage in Eurasian spruce bark beetle, Ipstypographus L.(Coleoptera: Curculionidae). Frontiers in Forests and Global Change, 6, 1176160.https://doi.org/10.3389/ffgc.2023.1176160

Chang, R., Duong, T. A., Taerum, S. J., Wingfield, M. J., Zhou, X., Yin, M., & De Beer, Z. W. (2019). Ophiostomatoid fungi associated with the spruce bark beetle Ipstypographus, including 11 new species from China. Persoonia-Molecular Phylogeny and Evolution of Fungi, 42(1), 50-74.https://doi.org/10.3767/persoonia.2019.42.03

Chen, T., Wang, Y., Dang, J., Zhang, F., & Wang, C. (2026). Detection technologies and sensing systems for crop pest identification and infestation severity prediction: a review. Frontiers in Plant Science, 17, 1765363.https://doi.org/10.3389/fpls.2026.1765363

Collier, R. (2023) Pest insect management in vegetable crops grown outdoors in northern Europeapproaches at the bottom of the IPM pyramid. Frontiers in Horticulture, 2, 1159375.https://doi.org/10.3389/fhort.2023.1159375

Chen, H., Li, Z., Bu, S. H., & Tian, Z. Q. (2011). Flight of the Chinese white pine beetle (Coleoptera: Scolytidae) in relation to sex, body weight and energy reserve. Bulletin of Entomological Research, 101(1), 53-62.https://doi.org/10.1017/S0007485310000209

Dacquin, P., Caiti, E., Grégoire, J. C., & Aron, S. (2024). Preemergence mating, inbreeding, and their consequences in the bark beetle Ips typographus. Journal of Pest Science, 97(2), 1005-1016. https://doi.org/10.1007/s10340-023-01650-4

Davis, T. S., Stewart, J. E., Mann, A., Bradley, C., & Hofstetter, R. W. (2019). Evidence for multiple ecological roles of Leptographiumabietinum, a symbiotic fungus associated with the North American spruce beetle. Fungal Ecology, 38, 62-70.https://doi.org/10.1016/j.funeco.2018.04.008

De Groot, M., Diaci, J., &Ogris, N. (2019). Forest management history is an important factor in bark beetle outbreaks: Lessons for the future. Forest Ecology and Management, 433, 467–474. https://doi.org/10.1016/j.foreco.2018.11.025

De Groot, M., Ogris, N., Diaci, J., & Castagneyrol, B. (2023). When tree diversity does not work: The interacting effects of tree diversity, altitude and amount of spruce on European spruce bark beetle outbreaks. Forest Ecology and Management, 537, 120952. https://doi.org/10.1016/j.foreco.2023.120952

Deganutti, L., Biscontin, F., Bernardinelli, I., Faccoli, M., 2024. The semiochemicalpushand-pull technique can reduce bark beetle damage in disturbed Norway spruce forests affected by the Vaia storm. Agric. For. Entomol. 26, 115–125. https://doi.

org/10.1111/afe.12600

Doležal, P., &Sehnal, F. (2007). Effects of photoperiod and temperature on the development and diapause of the bark beetle *Ips typographus*. Journal of Applied Entomology, 131(3), 165-173.https://doi.org/10.1111/j.1439-0418.2006.01123.x

EFSA - European Food Safety Authority. Panel on Plant Health (PLH), Jeger, M., Bragard, C., Caffier, D., Candresse, T., Chatzivassiliou, E., ... & Grégoire, J. C. (2017a). Pest categorisation of *Ips typographus*. EFSA journal, 15(7), e04881.https://doi.org/10.2903/j.efsa.2017.4881

EFSA Panel on Plant Health (PLH), Jeger, M., Bragard, C., Caffier, D., Candresse, T., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Jaques Miret, J. A., MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Rossi, V., Urek, G., Van Bruggen, A., Van der Werf, W., West, J., … Grégoire, J.

C. (2017b). Pest categorisation of *Ips duplicatus*. EFSA journal. European Food Safety Authority, 15(10), e05040. https://doi.org/10.2903/j.efsa.2017.5040

EFSA - European Food Safety Authority.Tramontini, S., Delbianco, A., & Vos, S. (2020). Pest survey card on Pissodescibriani, P. fasciatus, P. nemorensis, P. nitidus, P. punctatus, P. strobi, P. terminalis, P. yunnanensis and P. zitacuarense. EFSA Supporting Publications, 17(8), 1910E.https://doi.org/10.2903/sp.efsa.2020.EN-1910

EFSA (European Food Safety Authority), Rondoni G, Graziosi I, Mattion G, Carotti L, (2026). Pest survey card on *Ips amitinus*, *I. cembrae*, *I. duplicatus* and *I. sexdentatus*.https://doi.org/10.2903/sp.efsa.2026.EN-9885.

Eggleton, P., & Gaston, K. J. (1990). "Parasitoid" species and assemblages: Convenient definitions or misleading compromises?. Oikos, 417-421.https://doi.org/10.2307/3545155

El-sayed, A. M. (2026). The Pherobase: Database of Pheromones and Semiochemicals. Available in: http://www.pherobase.com/

EPPO - European and Mediterranean Plant Protection Organization. (2026) EPPO Global Database - *Ips typographus* (IPSXTY). Available in: https://gd.eppo.int/taxon/IPSXTY/documents

Erbilgin, N., Powell, J. S., & Raffa, K. F. (2003). Effect of varying monoterpene concentrations on the response of *Ips pini* (Coleoptera: Scolytidae) to its aggregation pheromone: implications for pest management and ecology of bark beetles. Agricultural and Forest Entomology, 5(4), 269-274.https://doi.org/10.1046/j.1461-9563.2003.00186.x

Erbilgin, N., Krokene, P., Kvamme, T., & Christiansen, E. (2007). A host monoterpene influences *Ips typographus* (Coleoptera: Curculionidae, Scolytinae) responses to its aggregation pheromone. Agricultural and Forest Entomology, 9, 135–140. https://doi.org/10.1111/j.1461-9563.2007.00329.x

European Commission:Energy, Climate change, Environment, in EU Actions.Available in: https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en

FAO - Food and Agriculture Organization of the United Nations. (2020). Guide to implementation of phytosanitary standards in forestry. FAO. https://www.fao.org/publications

FAO. Food and Agriculture Organization. Global Forest Resources Assessment 2025: Report Czechia. Food and Agriculture Organization of the United Nations Rome. Available in: https://openknowledge.fao.org/server/api/core/bitstreams/d81466fa-034d-4976-8217-a53ce8780ae5/content

Faccoli, M., & Schlyter, F. (2007). Conifer phenolic resistance markers are bark beetle antifeedant semiochemicals. Agricultural and Forest Entomology, 9(3), 237-245.https://doi.org/10.1111/j.1461-9563.2007.00339.x

Faccoli, M. (2009). Effect of weather on *Ips typographus* (Coleoptera: Curculionidae) phenology, voltinism, and associated spruce mortality in the southeastern Alps. Environmental Entomology, 38, 307–316. https://doi.org/10.1603/022.038.0202

Fält-Nardmann, J., Klemola, T., Roth, M., Ruohomäki, K., & Saikkonen, K. (2016). Northern geometrid forest pests (Lepidoptera: Geometridae) hatch at lower temperatures than their southern conspecifics: Implications of climate change. European Journal of Entomology, 113, 337.https://doi.org/10.14411/eje.2016.043

Fält-Nardmann, J. J., Klemola, T., Ruohomäki, K., Niemelä, P., Roth, M., & Saikkonen, K. (2018). Local adaptations and phenotypic plasticity may render gypsy moth and nun moth future pests in northern European boreal forests. CanadianJournal of Forest Research, 48(3), 265-276.https://doi.org/10.1139/cjfr-2016-0481

Fang, J., Shi, X., Du, H., Zhang, S., Liu, F., Liu, J., ... & Kong, X. (2026). Phytochemical signaling mediates host specificity and species interactions in the Eurasian spruce bark beetle. Journal of Pest Science, 99(1), 5. https://doi.org/10.1007/s10340-025-01977-0

Fernholz, K., Alderman, D., Novoselov, I., Palacin J. (2020). Economic Overview and Policies. Introduction and UNECE region overview: UNECE/FAO Forest Products Annual Market Review, 2019-2020. United Nation, Geneva. Available in: https://research.fs.usda.gov/download/treesearch/62346.pdf

Fettig, C. J., Asaro, C., Nowak, J. T., Dodds, K. J., Gandhi, K. J. K., Moan, J. E., & Robert, J. A. (2022a). Trends in bark beetle impacts in North America during a period (2000–2020) of rapid environmental change. Journal of Forestry, 120(6), 693–713. https://doi.org/10.1093/jofore/fvac021

Fettig, C. J., Egan, J. M., Delb, H., Hilszczański, J., Kautz, M., Munson, A. S., Nowak, J. T., & Negrón, J. F. (2022b). Management tactics to reduce bark beetle impacts in North America and Europe under altered forest and climatic conditions.

Fettig, C. J., Audley, J. P., & Munson, A. S. (2025). Applied chemical ecology ofspruce beetle in western North America. Forests, 16(7), 1103. https://doi.org/10.3390/f16071103

FISE– Forestry Information System for Europe. (2025). Reported damage symptoms per tree species in 2022. Available in: https://forest.eea.europa.eu/data/visualizations/damage-symptoms-by-agent-group-for-main-tree-species?activeTab=570bee2d-1316-48cf-adde-4b640f92119b

Flaherty, L., Gutowski, J. M. G., Hughes, C., Mayo, P., Mokrzycki, T., Pohl, G., ... & Sweeney, J. (2019). Pheromone-enhanced lure blends and multiple trap heights improve detection of bark and wood-boring beetles potentially moved in solid wood packaging. Journal of Pest Science, 92(1), 309-325.https://doi.org/10.1007/s10340-018-1019-4

Fleischer, P., Fereník, J., Hlavá, P., & Kozánek, M. (2016). Elevated bark temperature in unremoved stumps after disturbances facilitates multi-voltinism in Ipstypographus population in a mountainous forest. Central European Forestry Journal, 62, 15–22. https://doi.org/10.1515/forj-2016-0002

Foelker, C. J., & Hofstetter, R. W. (2014). Heritability, fecundity, and sexual size dimorphism in four species of bark beetles (Coleoptera: Curculionidae: Scolytinae). Annals of the Entomological Society of America, 107, 143–151. https://doi.org/10.1603/AN12153

Forzieri, G., Dutrieux, L. P., Elia, A., Eckhardt, B., Caudullo, G., Álvarez Taboada, F., ... & Cescatti, A. (2023). A Beck, PS, Giovanni Forzieri, C., and Commission, E.:

The Database of European Forest Insect and Disease Disturbances: DEFID2. Glob. Change Biol., https://doi. org/10.1111/gcb, 16912.

Franceschi, V. R., Krokene, P., Christiansen, E., &Krekling, T. (2005). Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytologist, 167, 353–376. https://doi.org/10.1111/j.1469-8137.2005.01436.x

Francke, W., &Vité, J. P. (1983). Oxygenated terpenes in pheromone systems of bark beetles 1. Zeitschrift für AngewandteEntomologie, 96(1-5), 146-156.https://doi.org/10.1111/j.1439-0418.1983.tb03655.x

Francke, W., Bartels, J., Meyer, H., Schröder, F., Kohnle, U., Baader, E., &Vité, J. P. (1995). Semiochemicals from bark beetles: new results, remarks, and reflections. Journal of Chemical Ecology, 21(7), 1043-1063.https://doi.org/10.1007/BF02033807

Franklin, A. J., & Grégoire, J. C. (2001). Dose-dependent response and preliminary observations on attraction range of *Ips typographus* to pheromones at low release rates. Journal of chemical ecology, 27(12), 2425-2435.https://doi.org/10.1023/A:1013619313415

Frühbrodt, T., Du, B., Delb, H., Burzlaff, T., Kreuzwieser, J., & Biedermann, P. H. W. (2023). Know when you are too many: Density-dependent release of pheromones during host colonisation by the European spruce bark beetle, *Ips typographus* (L.). Journal of Chemical Ecology, 49, 652–665.https://doi.org/10.1007/s10886-023-01453-y

Frühbrodt, T., Löcken, H., Du, B., Fettig, C. J., Biedermann, P. H. W., Kreuzwieser, J., Burzlaff, T., &Delb, H. (2024a). Verbenone (SPLAT® Verb) delays *Ips typographus* (L.) infestation and reduces infestation risk and severity in windthrown Norway spruce in Southwest Germany. Forest Ecology and Management, 561, 121856. https://doi.org/10.1016/j.foreco.2024.121856

Frühbrodt, T., Schebeck, M., Andersson, M. N., Holighaus, G., Kreuzwieser, J., Burzlaff, T., Delb, H., & Biedermann, P. H. W. (2024b). Verbenone—The universal bark beetle repellent? Its origin, effects, and ecological roles. Journal of Pest Science, 97, 35–71. https://doi.org/10.1007/s10340-023-01635-3

Gandhi, K. J. K., & Hofstetter, R. W. (Eds.). (2021). Bark beetle management, ecology, and climate change. Academic Press. https://doi.org/10.1016/C2019-0-04867-3

Ganji, S., Svensson, F. G., & Unelius, C. R. (2020). Asymmetric synthesis of oxygenated monoterpenoids of importance for bark beetle ecology. Journal of natural products, 83(11), 3332-3337.https://doi.org/10.1021/acs.jnatprod.0c00669

Gomez, D. F., Riggins, J. J., & Cognato, A. I. (2023). Bark beetles. In Forestentomology and pathology: volume 1: entomology (pp. 299-337). Cham: Springer International Publishing.https://doi.org/10.1007/978-3-031-11553-0_10

Grodzki, W. (2004). Some reactions of *Ips typographus* (L.) (Col.: Scolytidae) to changing breeding conditions in a forest decline area in the Sudeten Mountains. Journal of Pest Science, 77, 43-48. https://doi.org/10.1007/s10340-003-0026-1

Hallas, T., Hoch, G., Kirisits, T., & Netherer, S. (2026). Assessing disturbance risks of the European spruce bark beetle under climate change: a review of model approaches and future perspectives. European Journal of Forest Research, 145(2), 23.https://doi.org/10.1007/s10342-025-01841-x

Hansson, B. S., &Stensmyr, M. C. (2011). Evolution of insect olfaction. Neuron, 72(5), 698-711. https://doi.org/10.1016/j.neuron.2011.11.003

Harring, C. M., & Mori, K. (1976). Pityokteinescurvidens Germ.(Coleoptera: Scolytidae): Aggregation in response to optically pure ipsenol 1. Zeitschrift für AngewandteEntomologie, 82(1-4), 327-329.

Harring, C. M. (1978). Aggregation pheromones of the European fir engraver beetles Pityokteinescurvidens, P. spinidens and P. vorontzovi and the role of juvenile hormone in pheromone biosynthesis 1. Zeitschrift für AngewandteEntomologie, 85(1-4), 281-317.https://doi.org/10.1111/j.1439-0418.1978.tb04040.x

Heber, T., Helbig, C. E., Osmers, S., & Müller, M. G. (2021). Evaluation of attractant composition, application rate, and trap type for potential mass trapping of *Ips typographus* (L.). Forests, 12(12), Article 1727. https://doi.org/10.3390/f12121727

Henning, A. P., & Hofstetter, R. W. (2025). A novel choice test to detect the influence of fungi on the tunnelingbehavior of sympatric bark beetles (Coleoptera: Scolytinae). Environmental Entomology, 54(4), 819-827.

Herms, D. A., Haack, R. A., & Ayres, B. D. (1991). Variation in semiochemical-mediated prey-predator interaction: *Ips pini* (Scolytidae) and *Thanasimus dubius* (Cleridae). Journal of Chemical Ecology, 17(8), 1705-1714.https://doi.org/10.1007/BF00984698

Heuer, H. G., and Vité, J. P. (1984). Chalcogran: Unique kairomone-governed predator-prey relations among Ostomid and Scolytid beetles. https://doi.org/10.1007/BF00490438

Hayes, J. L., & Strom, B. L. (1994). 4-Allylanisole as an inhibitor of bark beetle (Coleoptera: Scolytidae) aggregation. Journal of Economic Entomology, 87(6), 1586-1594. https://doi.org/10.1093/jee/87.6.1586

Hilszczański, J., Gibb, H., &Bystrowski, C. (2007). Insect natural enemies of *Ips typographus* (L.) (Coleoptera, Scolytinae) in managed and unmanaged stands of mixed lowland forest in Poland. Journal of Pest Science. https://doi.org/10.1007/s10340-006-0160-7

Hofmann, S., Schebeck, M., & Kautz, M. (2024). Diurnal temperature fluctuations improve predictions of developmental rates in the spruce bark beetle *Ips typographus*. Journal of Pest Science, 97(4), 1839-1852.https://doi.org/10.1007/s10340-024-01758-1

Holuša, J., Lukášová, K., & Lubojacký, J. (2012). Comparison of seasonal flight activity of *Ips typographus* and *Ips duplicatus*. Scientia agriculturae bohemica, 43(3), 109-115.

Hou, X. Q., Yuvaraj, J. K., Roberts, R. E., Zhang, D. D., Unelius, C. R., Löfstedt, C., & Andersson, M. N. (2021). Functional evolution of a bark beetle odorant receptor clade detecting monoterpenoids of different ecological origins. Molecular biology and evolution, 38(11), 4934-4947.https://doi.org/10.1093/molbev/msab218

Hlásny, T., Krokene, P., Liebhold, A., Montagné-Huck, C., Müller, J., Qin, H., Raffa, K., Schelhaas, M., Seidl, R. & Svoboda, M. (2019) Living with bark beetles:

impacts, outlook and management options. From Science to Policy 8. European Forest Institute.

Hlásny, T., Zimová, S., Merganičová, K., Štěpánek, P., Modlinger, R., & Turčáni, M. (2021). Devastating outbreak of bark beetles in the Czech Republic: Drivers, impacts, and 133 management implications. Forest Ecology and Management, 490, 119075. https://doi.org/10.1016/j.foreco.2021.119075

Hlásny, T., König, L., Krokene, P., Lindner, M., Montagné-Huck, C., Müller, J., Qin, H., Raffa, K. F., Schelhaas, M.-J., Svoboda, M., Viiri, H., & Seidl, R. (2021b). Bark Beetle Outbreaks in Europe: State of Knowledge and Ways Forward for Management. Current Forestry Reports, 7(3), 138–165. https://doi.org/10.1007/s40725-021-00142-x

Hroššo, B., Mezei, P., Potterf, M., Majdák, A., Blazenec, M., Korolyova, N. & Jakub, R. (2020) Drivers of spruce bark beetle (*Ips typographus*) infestations on downed trees after severe windthrow. Forests, 11, 1290.https://doi.org/10.3390/f11121290

Hulcr, J., Ubik, K., &Vrkoc, J. (2006). The role of semiochemicals in tritrophic interactions between the spruce bark beetle *Ips typographus*, its predators and infested spruce. Journal of Applied Entomology, 130(5), 275-283.https://doi.org/10.1111/j.1439-0418.2006.01069.x

Hulcr, J., Pollet, M., Ubik, K., &Vrkoč, J. (2013). Exploitation of kairomones and synomones by Medetera spp.(Diptera: Dolichopodidae), predators of spruce bark beetles. European Journal of Entomology, 102(4), 655-662.

Hulcr, J., Atkinson, T. H., Cognato, A. I., Jordal, B. H., & McKenna, D. D. (2015). Morphology, taxonomy, and phylogenetics of bark beetles. In F. E. Vega & R. W. Hofstetter (Eds.), Bark beetles: Biology and ecology of native and invasive species (pp. 41–84). Academic Press. https://doi.org/10.1016/B978-0-12-417156-5.00002-4

Hulcr, J., &Stelinski, L. L. (2017). The ambrosia symbiosis: From evolutionary ecology to practical management. Annual Review of Entomology, 62, 285–303. https://doi.org/10.1146/annurev-ento-031616-035105

Hüttnerová, T., Paczkowski, S., Neubert, T., Jirošová, A., & Surový, P. (2023). Comparison of individual sensors in the electronic nose for stress detection in forest stands. Sensors, 23(4), 2001.https://doi.org/10.3390/s23042001

Hüttnerová, T., & Surový, P. (2024). Bark beetle detection method using electronic nose sensors. A possible improvement of early forest disturbance detection?. Frontiers in Forests and Global Change, 7, 1445094.https://doi.org/10.3389/ffgc.2024.1445094

IPPC (2023) Climate Change 2023: Synthesis Report, Summary for Policymakers. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland

Jaakkola, E., Gärtner, A., Jönsson, A. M., Olsson, K. L. P., & Holst, T. (2023). Spruce bark beetle (*Ips typographus*) infestation causes up to 700 times higher bark BVOC emission rates from Norway spruce (*Picea abies*). Biogeosciences, 20, 803–826. https://doi.org/10.5194/bg-20-803-2023

Jacquin-Joly, E., & Merlin, C. (2004). Insect olfactory receptors: contributions of molecular biology to chemical ecology. Journal of chemical ecology, 30(12), 2359-2397.https://doi.org/10.1007/s10886-004-7941-3

Jakoby, O., Lischke, H. &Wermelinger, B. (2019) Climate change alters elevational phenology patterns of the European spruce bark beetle (*Ips typographus*). Global Change Biology, 25, 4048-4063. https://doi.org/10.1111/gcb.14766

Jakuš, R., Modlinger, R., Kašpar, J., Majdak, A., Blaženec, M., Korolyova, N., & Schlyter, F. (2022). Testing the efficiency of the push-and-pull strategy during severe *Ips typographus* outbreak and extreme drought in Norway spruce stands. Forests, 13(12), 2175. https://doi.org/10.3390/f13122175

Jakuš R, Trubin A, Singh VV, Zabihi K, Jirošová A, Modlinger R, Majdák A, Korolyova N, Moliterno AAC, Kašpar J, Slavík M. Spruce protection against *Ips typographus* with anti-attractant blend of tree-based semiochemicals: from small experimental plots to stand scales. Forests. 2024; 15(2), p.356; 2024; doi:10.3390/f15020356

Jakuš, R., Trubin, A., Singh, V. V., Zabihi, K., Jirošová, A., Modlinger, R., & Schlyter, F. (2024). Spruce protection against *Ips typographus* with anti-attractant blend of tree-based semiochemicals: From small experimental plots to stand scales. Forests, 15(2), 356. https://doi.org/10.3390/f15020356

Jirošová, A., Kalinová, B., Modlinger, R., Jakuš, R., Unelius, C. R., Blaženec, M., & Schlyter, F. (2022). Anti-attractant activity of (+)-trans-4-thujanol for Eurasian spruce bark beetle *Ips typographus*: Novel potency for females. Pest Management Science, 78(5), 1992–1999. https://doi.org/10.1002/ps.6819

Jordal, B. H. (2014). Scolytinae Latreille, 1804. In R. A. B. Leschen& R. G. Beutel (Eds.), Handbook of zoology: Arthropoda: Insecta: Coleoptera, beetles. Volume 3: Morphology and systematics (Phytophaga) (pp. 633–642). De Gruyter. https://doi.org/10.1515/9783110274462.633

Johny, J., Große-Wilde, E., Kalinová, B., & Roy, A. (2024). Antennal transcriptome screening and identification of chemosensory proteins in the double-spine European spruce bark beetle, *Ips duplicatus* (Coleoptera: Scolytinae). International Journal of Molecular Sciences, 25(17), 9513.https://doi.org/10.3390/ijms25179513

Johny J, Diallo S, Nadachowska-Brzyska K, Moliterno AAC, Roy A, Kalinová B, Große-Wilde E, Schlyter F. (2025). Not all bark beetles smell the same: population-level functional olfactory polymorphisms in *Ips typographus* pheromone receptor ItypOR33. Molecular Ecology, 34(6), p.e17693; https://doi.org/10.1111/mec.17693

Joseph, G., Kelsey, R. G., Peck, R. W., & Niwa, C. G. (2001). Response of some scolytids and their predators to ethanol and 4-allylanisole in pine forests of central Oregon. Journal of Chemical Ecology, 27(4), 697-715. https://doi.org/10.1023/A:1010345817756

Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, S., & Denno, R. F. (2008). Constitutive and induced defenses to herbivory in above-and belowground plant tissues. Ecology, 89(2), 392-406.https://doi.org/10.1890/07-0471.1

Kausrud, K., Økland, B., Skarpaas, O., Grégoire, J.-C., Erbilgin, N., & Stenseth, N. C. (2012). Population dynamics in changing environments: The case of an eruptive forest pest species. Biological Reviews, 87(1), 34–51. https://doi.org/10.1111/j.1469-185X.2011.00183.x

Kalinová, B., Břízová, R., Knížek, M., Turčáni, M., & Hoskovec, M. (2014). Volatiles from spruce trap-trees detected by *Ips typographus* bark beetles: Chemical and electrophysiological analyses. Arthropod-Plant Interactions, 8, 305–316. https://doi.org/10.1007/s11829-014-9310-7

Kandasamy, D., Gershenzon, J., & Hammerbacher, A. (2016). Volatile organic compounds emitted by fungal associates of conifer bark beetles and their potential in bark beetle control. Journal of Chemical Ecology, 42(9), 952-969.https://doi.org/10.1007/s10886-016-0768-x

Kandasamy, D., Gershenzon, J., Andersson, M. N., & Hammerbacher, A. (2019). Volatile organic compounds influence the interaction of the Eurasian spruce bark beetle (*Ips typographus*) with its fungal symbionts. The ISME journal, 13(7), 1788-1800. https://doi.org/10.1038/s41396-019-0390-3

Kandasamy, D., Zaman, R., Nakamura, Y., Zhao, T., Hartmann, H., Andersson, M. N., Hammerbacher, A., & Gershenzon, J. (2023). Conifer-killing bark beetles locate fungal symbionts by detecting volatile fungal metabolites of host tree resin monoterpenoids. PLoS Biology, 21, e3001887. https://doi.org/10.1371/journal.pbio.3001887

Kenis, M., Wegensteiner, R., & Griffin, C. T. (2004). Parasitoids, predators, nematodes and pathogens associated with bark weevil pests. In F. Lieutier, K. R. Day, A. Battisti, J.-C. Grégoire, & H. F. Evans (Eds.), Bark and wood boring insects in living trees in Europe, a synthesis. Springer. https://doi.org/10.1007/978-1-4020-2241-8_18

KirisitsT. (2007). Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi: in Bark and wood boring insects in living trees in Europe, a synthesis, edsLieutierF.DayK. R.BattistiA.GrégoireJ. C.EvansH. F. (Dordrecht: Springer), 181–236.

Köhl, M., Koch, F. H., Linser, S., Melin, M., Robertson, G., & Talarczyk, A. (2024). Reporting on forest damages and disturbances in the UNECE region.Available in: https://research.fs.usda.gov/treesearch/68027

Kopper, B. J., Klepzig, K. D., & Raffa, K. F. (2004). Components of antagonism and mutualism in *Ips pini*–fungal interactions: relationship to a life history of colonizing highly stressed and dead trees. Environmental Entomology, 33(1), 28-34.https://doi.org/10.1603/0046-225X-33.1.28

Kopper, B. J., Illman, B. L., Kersten, P. J., Klepzig, K. D., & Raffa, K. F. (2005). Effects of diterpene acids on components of a conifer bark beetle–fungal interaction: tolerance by *Ips pini* and sensitivity by its associate Ophiostomaips. Environmental entomology, 34(2), 486-493.https://doi.org/10.1603/0046-225X-34.2.486

Korolyova, N., Deganutti, L., Majdák, A., Trubin, A., Basile, S., Jakuš, R., & Faccoli, M. (2026). The paradox of attraction: Why push-and-pull doesn't always work for spruce protection against bark beetles. Forest Ecology and Management, 614, 123804.https://doi.org/10.1016/j.foreco.2026.123804

Kula, E., & Hrdlička, P. (2025). Behaviour of European spruce bark beetle—*Ips typographus* (L.) on poisoned traps. Journal of Applied Entomology, 149, 1279–1291. https://doi.org/10.1111/jen.13454

Kuhn, A., Hautier, L., & San Martin, G. (2022). Do pheromone traps help to reduce new attacks of *Ips typographus* at the local scale after a sanitary cut? PeerJ, 10, Article e14093. https://doi.org/10.7717/peerj.14093

KnÍŽek, M., & Beaver, R. (2007). Taxonomy and systematics of bark and ambrosia beetles. In Bark and wood boring insects in living trees in Europe, a synthesis (pp. 41-54). Dordrecht: Springer Netherlands.https://doi.org/10.1007/978-1-4020-2241-8_5

La Porta, N. I. C. O. L. A., Capretti, P. I. M. T., Thomsen, I. M., Kasanen, R., Hietala, A. M., & Von Weissenberg, K. (2008). Forest pathogens with higher damage potential due to climate change in Europe. Canadian Journal of Plant Pathology, 30(2), 177-195.https://doi.org/10.1080/07060661.2008.10540534

Lehmanski, L. M., Kandasamy, D., Andersson, M. N., Netherer, S., Alves, E. G., Huang, J., et al. (2023). Addressing a century old hypothesis—Do pioneer beetles of *Ips typographus* use volatile cues to find suitable host trees? New Phytologist, 238, 1762–1770. https://doi.org/10.1111/nph.18865

Leigh, G. J. (Ed.). (2011). Principles of chemical nomenclature: a guide to IUPAC recommendations. Royal Society of Chemistry.

Lieutier, F., Day, K. R., Battisti, A., Grégoire, J.-C., & Evans, H. F. (2.Eds.). (2007). Bark and wood boring insects in living trees in Europe: A synthesis. Kluwer Academic Publishers. https://doi.org/10.1007/978-1-4020-2241-8

Lieutier, F., Yart, A., & Salle, A. (2009). Stimulation of tree defenses by Ophiostomatoid fungi can explain attack success of bark beetles on conifers. Annals of Forest Science, 66(8), 801-801.https://doi.org/10.1051/forest/

Lindmark, M., Wallin, E. A., & Jonsson, B.-G. (2022). Protecting forest edges using trap logs: Limited effects of associated push-pull strategies targeting *Ips typographus*. ForestEcology and Management, 505, Article 119886. https://doi.org/10.1016/j.foreco.2021.119886

Linnakoski, R., De Beer, Z. W., Niemelä, P., & Wingfield, M. J. (2012). Associations of conifer-infesting bark beetles and fungi in Fennoscandia. Insects, 3(1), 200-227.

https://doi.org/10.3390/insects3010200

Lobinger, G. (1994) Air temperature as a limiting factor for flight activity of two species of pine bark beetles, *Ips typographus* L. and Pityogeneschalcographus L.(Col., Scolytidae). Journal of Pest Science, 67, 14-172009066

Lubojacký, J., & Holuša, J. (2011). Comparison of spruce bark beetle (*Ips typographus*) catches between treated trap logs and pheromone traps. Šumarski List, 135(5–6), 233–242.

Lubojácký, J., Lorenc, F., Véle, A., Knížek, M., & Liška, J. (2022). Výskytlesníchškodlivýchfaktorů v Česku v roce 2022. Zpravodaj: Škodlivíčinitelé v lesíchČeska 109 2021/2022–Škodyzvěří. Sborníkreferátů z celostátníhoseminář e s mezinárodníúčastí, 6, 17–26.

Macías-Sámano, J. E., Borden, J. H., Pierce Jr, H. D., Gries, R., & Gries, G. (1997). Aggregation pheromone of Pityokteines elegans. Journal of chemical ecology, 23(5), 1333-1347.https://doi.org/10.1023/B:JOEC.0000006467.79265.3c

Martin J.P., Beyerlein A., Dacks A.M, Reisenman C.E., Riffell J.A, Lei H, Hildebrand J.G.(2011). The neurobiology of insect olfaction: sensory processing in a comparative context. Journal of Progress in Neurobiology. 95:427447. https://doi.org/10.1016/j.pneurobio.2011.09.007

MacLean, D. A. (2016). Impacts of insect outbreaks on tree mortality, productivity, and stand development. The Canadian Entomologist, 148(S1), S138-S159.https://doi.org/10.4039/tce.2015.24

McTavish, C. K., Catal, M. Ü. R. S. E. L., Fulbright, D. W., & Jarosz, A. M. (2018). Spruce decline and Diaporthe: incidence, taxonomy, virulence, and tree susceptibility in Michigan. Plant disease, 102(11), 2330-2340.https://doi.org/10.1094/PDIS-06-17-0926-RE

Mendel, Z. (1988). Attraction of Orthotomicuserosus and Pityogenescalcaratus to a synthetic aggregation pheromone of *Ips typographus*. Phytoparasitica, 16(2), 109-117.

Mezei, P., Potterf, M., Škvarenina, J., Rasmussen, J. G., & Jakuš, R. (2019). Potential solar radiation as a driver for bark beetle infestation on a landscape scale. Forests, 10(7), 604.https://doi.org/10.3390/f10070604

Michel A, Haggenmüller K, Kirchner T, Prescher A-K, Schwärzel K, Wohlgemuth L, editors. (2024). Forest Condition in Europe:The 2024 Assessment. ICP Forests Technical Report under the UNECE Convention on Long-range Transboundary Air

Pollution (Air Convention). Eberswalde: Thünen Institute. 96 p. https://doi.org/10.3220/ICPTR1732702585000

Miścicki, S., & Grodzki, W. (2021). Can sanitation cutting contribute to reduced mortality of Norway spruce *Picea abies* (L.) H. Karst., due to infestation by *Ips typographus* (L.)? Sylwan, 165(11), 749–762. https://doi.org/10.26202/sylwan.2021105

Missbach, C., Dweck, H. K., Vogel, H., Vilcinskas, A., Stensmyr, M. C., Hansson, B. S., & Grosse-Wilde, E. (2014). Evolution of insect olfactory receptors. elife, 3, e02115. https://doi.org/10.7554/eLife.02115

Miller, D. R., & Borden, J. H. (1990). The use of monoterpenes as kairomones by *Ips latidens* (LeConte)(Coleoptera: Scolytidae). The Canadian Entomologist, 122(2), 301-307.https://doi.org/10.4039/Ent122301-3

Miller, D. R., Borden, J. H., & Lindgren, B. S. (1995). Verbenone: dose-dependent interruption of pheromone-based attraction of three sympatric species of pine bark beetles (Coleoptera: Scolytidae). Environmental entomology, 24(3), 692-696.https://doi.org/10.1093/ee/24.3.692

Miller, D. R., Borden, J. H., & Slessor, K. N. (1996). Enantiospecific pheromone production and response profiles for populations of pine engraver, *Ips pini* (Say)(Coleoptera: Scolytidae), in British Columbia. Journal of chemical ecology, 22(11), 2157-2172.https://doi.org/10.1007/BF02040100

Miller, D. R., & Borden, J. H. (2003). Responses of *Ips pini* (Say), Pityogenesknechteli Swaine and associated beetles (Coleoptera) to host monoterpenes in stands of lodgepole pine. Journal of Entomological Science, 38(4), 602-611. https://doi.org/10.18474/0749-8004-38.4.602

Modlinger, R., & ka, L. J. (2016). Review of Lepidoptera with trophic relationships to *Picea abies* (L.) in the conditions of Czechia. Central European Forestry Journal, 62(4), 178-194.https://doi.org/10.1515/forj-2016-0015

Moliterno, A. A. C., Jakuš, R., Modlinger, R., Unelius, C. R., Schlyter, F., & Jirošová, A. (2023). Field effects of oxygenated monoterpenoids and estragole combined with pheromone on attraction of *Ips typographus* and its natural enemies. Frontiers in Forests and Global Change, 6, 1292581. https://doi.org/10.3389/ffgc.2023.1292581

Moliterno, A. A. C., Shewale, M. K., Basile, S., Synek, J., & Jirošová, A. (2025). Size-dependent behavioural and antennal responses to doses of (+)-isopinocamphone and 1, 8-cineole mixed with pheromone: a potential host

selection strategy in female *Ips typographus* L. Annals of Forest Science, 82(1), 34.https://doi.org/10.1186/s13595-025-01297-4

Moore, G. E. (1972). Southern pine beetle mortality in North Carolina caused by parasites and predators. Environmental Entomology, 1(1), 58-65. https://academic.oup.com/ee/article-abstract/1/1/58/2392522

Mori, K. (2007). Significance of chirality in pheromone science. Bioorganic & Medicinal Chemistry, 15(24), 7505-7523.https://doi.org/10.1016/j.bmc.2007.08.040

Munro, H. L., Gandhi, K. J., Barnes, B. F., Montes, C. R., Nowak, J. T., Shepherd, W. P., ... & Sullivan, B. T. (2020). Electrophysiological and behavioral responses Dendroctonus frontalis and D. terebrans (Coleoptera: Curculionidae) to resin odors of host pines (Pinus spp.) HL Munro et al. Chemoecology, 30(5), 215-231. https://doi.org/10.1007/s00049-020-00311-7

Netherer, S., Panassiti, B., Pennerstorfer, J. & Matthews, B. (2019) Acute drought is an important driver of bark beetle infestation in Austrian Norway spruce stands. Frontiers in Forests and Global Change, 2, 39.https://doi.org/10.3389/ffgc.2019.00039

Netherer, S., Kandasamy, D., Jirosová, A., Kalinová, B., Schebeck, M., & Schlyter, F. (2021). Interactions among Norway spruce, the bark beetle *Ips typographus* and its fungal symbionts in times of drought. Journal of Pest Science, 94(3), 591-614.https://doi.org/10.1007/s10340-021-01341-y

Netherer, S., & Hammerbacher, A. (2022). The Eurasian spruce bark beetle in a warming climate: Phenology, behavior, and biotic interactions. In K. J. K. Gandhi & R. W. Hofstetter (Eds.), Bark beetle management, ecology, and climate change (pp. 89–131). Academic Press.

Netherer, S. (2023). Towards an improved understanding of bark beetle and other insect herbivore infestation in conifer forests. Die Bodenkultur: Journal of Land Management, Food and Environment, 73(2), 135-151.https://doi.org/10.2478/boku-2022-0010

Netherer, S., Lehmanski, L., Bachlehner, A., Rosner, S., Savi, T., Schmidt, A., ... & Gershenzon, J. (2024). Drought increases Norway spruce susceptibility to the Eurasian spruce bark beetle and its associated fungi. New Phytologist, 242(3), 1000-1017.https://doi.org/10.1111/nph.19635

Økland, B., Netherer, S., & Marini, L. (2015). The Eurasian spruce bark beetle: The role of climate. In C. Björkman & P. Niemelä (Eds.), Climate change and insect pests (pp. 202–219). CABI. https://doi.org/10.1079/9781780643786.0202

Ogris, N., Ferlan, M., Hauptman, T., Pavlin, R., Kavi, A., Jurc, M. & De Groot, M. (2019). RITY3A phenology model of *Ips typographus* as a tool for optimization of its monitoring. Ecological Modelling, 410, 108775. https://doi.org/10.1016/j.ecolmodel.2019.108775

Paynter, Q. E., Anderbrant, O., & Schlyter, F. (1990). Behavior of male and female spruce bark beetles, Ips typographus, on the bark of host trees during mass attack. Journal of Insect Behavior, 3(4), 529-543. https://doi.org/10.1007/BF01052016

Pettersson, E. M., Hallberg, E., & Birgersson, G. (2001a). Evidence for the importance of odour-perception in the parasitoid*Rhopalicus tutela* (Walker)(Hym., Pteromalidae). Journal of Applied Entomology, 125(6), 293-301.https://doi.org/10.1046/j.1439-0418.2001.00550.x

Pettersson, E. M. (2001). Volatile attractants for three Pteromalid parasitoids attacking concealed spruce bark beetles. Chemoecology, 11(2), 89-95.https://doi.org/10.1007/PL00001837

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

Potterf, M., Frühbrodt, T., Thom, D., Lemme, H., Hahn, A., & Seidl, R. (2025). Hotter drought increases population levels and accelerates phenology of the European spruce bark beetle *Ips typographus*. Forest Ecology and Management, 585, 122615. https://doi.org/10.1016/j.foreco.2025.122615

Poland, T. M., & Borden, J. H. (1997). Attraction of a bark beetle predator, Thanasimusundatulus (Coleoptera: Cleridae), to pheromones of the spruce beetle and two secondary bark beetles (Coleoptera: Scolytidae). Journal of the Entomological Society of British Columbia, 94, 35-42.

Pureswaran, D. S., & Borden, J. H. (2003). Is bigger better? Size and pheromone production in the mountain pine beetle, Dendroctonus ponderosae Hopkins (Coleoptera: Scolytidae). Journal of Insect Behavior, 16, 765–782. https://doi.org/10.1023/B:JOIR.0000018319.37649.c4

Raffa, K. F., & Dahlsten, D. L. (1995). Differential responses among natural enemies and prey to bark beetle pheromones. Oecologia. https://doi.org/10.1007/BF00333305

Raffa, K. F., & Berryman, A. A. (1983). The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). Ecological monographs, 53(1), 27-49.https://doi.org/10.2307/1942586

Raffa, K. F. (2001). Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. Chemoecology, 11(2), 49-65. https://doi.org/10.1007/PL00001833

Raffa, K. F. (2014). Terpenes tell different tales at different scales: Glimpses into the chemical ecology of conifer–bark beetle–microbial interactions. Journal of Chemical Ecology, 40, 1–20. https://doi.org/10.1007/s10886-013-0368-y

Raffa, K. F., Aukema, B. H., Bentz, B. J., Carroll, A. L., Hicke, J. A., Kolb, T. E., & Lundquist, J. E. (2015). Responses of tree-killing bark beetles to a changing climate. In C. Björkman & P. Niemelä (Eds.), Climate change and insect pests (pp. 173–201). CABI. https://doi.org/10.1079/9781780643786.0173

Raffa, K. F., Andersson, M. N., & Schlyter, F. (2016). Host selection by bark beetles: Playing the odds in a high-stakes game. Advances in Insect Physiology, 50, 1–74. https://doi.org/10.1016/bs.aiip.2016.02.001

Ramakrishnan, R., Hradecký, J., Roy, A., Kalinová, B., Mendezes, R. C., Synek, J., ... & Jirošová, A. (2022). Metabolomics and transcriptomics of pheromone

biosynthesis in an aggressive forest pest *Ips typographus*. Insect Biochemistry and Molecular Biology, 140, 103680.https://doi.org/10.1016/j.ibmb.2021.103680

Ramakrishnan, R., Roy, A., Hradecký, J., Kai, M., Harant, K., Svatoš, A., & Jirošová, A. (2024). Juvenile hormone III induction reveals key genes in general metabolism, pheromone biosynthesis, and detoxification in Eurasian spruce bark beetle. Frontiers in Forests and Global Change, 6, 1215813.https://doi.org/10.3389/ffgc.2023.1215813

Ramakrishnan, R., Shewale, M. K., Strádal, J., Frühbrodt, T., Doležal, P., Andersson, M. N., & Jirošová, A. (2025). Aggregation pheromones in the bark beetle genus Ips: Advances in biosynthesis, sensory detection, and forest management applications. Current Forestry Reports, 11(1), 21. DOI: not provided in supplied text / verify.

Ramírez-Reyes, T., Armendáriz-Toledano, F., & Rodríguez, L. G. C. (2023). Rearranging and completing the puzzle: phylogenomic analysis of bark beetles Dendroctonus reveals new hypotheses about genus diversification. Molecular Phylogenetics and Evolution, 187, 107885.https://doi.org/10.1016/j.ympev.2023.107885

Reeve, J. D. (1997). Predation and bark beetle dynamics. Oecologia. https://doi.org/10.1007/s004420050282

Reeve, J. D., Rojas, M. G., & Morales-Ramos, J. A. (2003). Artificial diet and rearing methods for *Thanasimus dubius* (Coleoptera: Cleridae), a predator of bark beetles (Coleoptera: Scolytidae). Biological Control. https://www.sciencedirect.com/science/article/pii/S1049964403000215

Romeiro, J. M. N., Gohli, J., Krokene, P., Eid, T., & Antón Fernández, C. (2024). Bark beetle damage in Norwegian forests: A study of model suitability and projected impact under climate change. Scandinavian Journal of Forest Research, 39(1), 30–43. https://doi.org/10.1080/02827581.2023.2289648

Rudinsky, J. A., Novak, V., &Švihra, P. (1971). Attraction of the bark beetle Ipstypographus L. to terpenes and a male-produced pheromone. Journal of Applied Entomology, 67, 179–188. DOI: not provided in supplied text / verify.

Sallé, A., Baylac, M., &Lieutier, F. (2005). Size and shape changes of *Ips typographus* L.(Coleoptera: Scolytinae) in relation to population level. Agricultural and Forest Entomology, 7(4), 297-306.https://doi.org/10.1111/j.1461-9555.2005.00274.x

Sallé, A., & Raffa, K. F. (2007). Interactions among intraspecific competition, emergence patterns, and host selection behaviour in *Ips pini* (Coleoptera: Scolytinae). Ecological Entomology, 32(2), 162-171.https://doi.org/10.1111/j.1365-2311.2006.00833.x

Savoie, A., Borden, J. H., Pierce Jr, H. D., Gries, R., & Gries, G. (1998). Aggregation pheromone of Pityogenesknechteli and semiochemical-based interactions with three other bark beetles. Journal of Chemical Ecology, 24(2), 321-337.https://doi.org/10.1023/A:1022536526161

Seybold, S. J., Ohtsuka, T., Wood, D. L., & Kubo, I. (1995). Enantiomeric composition of ipsdienol: a chemotaxonomic character for North American populations of Ips spp. in the pini subgeneric group (Coleoptera: Scolytidae). Journal of Chemical Ecology, 21(7), 995-1016. https://doi.org/10.1007/BF02033804

Seybold, S. J., Bentz, B. J., Fettig, C. J., Lundquist, J. E., Progar, R. A., & Gillette, N. E. (2018). Management of western North American bark beetles with semiochemicals. Annual Review of Entomology, 63, 407-432.https://doi.org/10.1146/annurev-ento-020117-043339

Sierota, Z., & Grodzki, W. (2020). *Picea abies*–Armillaria–Ips: A strategy or coincidence?. Forests, 11(9), 1023.https://doi.org/10.3390/f11091023

Six, D. L. (2003). Bark beetle-fungus symbioses. In: Insect symbiosis, 1, 97-114.

Six, D. L., Hammerbacher, A., Trowbridge, A., & Bullington, L. (2026). From beginning to end: the synecology of tree-killing bark beetles, fungi, and trees. Biological Reviews, 101(1), 314-335.https://doi.org/10.1111/brv.70084

Shewale, M. K., Bláha, J., Synek, J., Schebeck, M., Andersson, M. N., Kandasamy, D., & Jirošová, A. (2025). Comparative analysis of olfactory sensory neurons in two Ips species reveals conserved and species-specific olfactory adaptations.

Frontiers in Forests and Global Change, 8, 1588866.https://doi.org/10.3389/ffgc.2025.1588866

Schebeck, M., Schopf, A., Ragland, G., Stauffer, C. & Biedermann, P. (2023) Evolutionary ecology of the bark beetles *Ips typographus* and Pityogeneschalcographus. Bulletin of Entomological Research, 113, 1-10.https://doi.org/10.1017/S0007485321000353

Schiebe, C., Blaženec, M., Jakuš, R., Unelius, C. R., & Schlyter, F. (2011). Semiochemical diversity diverts bark beetle attacks from Norway spruce edges. Journal of Applied Entomology, 135, 726–737. https://doi.org/10.1111/j.1439-0418.2011.01624.x

Schiebe, C., Unelius, C. R., Ganji, S., Binyameen, M., Birgersson, G., & Schlyter, F. (2019). Styrene, (+)-trans-(1R,4S,5S)-4-thujanol and oxygenated monoterpenes relatedto host stress elicit strong electrophysiological responses in the bark beetle Ipstypographus. Journal of Chemical Ecology, 45(5), 474–489. DOI: not provided in supplied text / verify.

Schlyter, F., &Löfqvist, J. (1986). Response of walking spruce bark beetles *Ips typographus* to pheromone produced in different attack phases. EntomologiaExperimentalis et Applicata, 41, 219–230. DOI: not provided in supplied text / verify.

Schlyter, F., Byers, J. A., &Löfqvist, J. (1987). Attraction to pheromone sources of different quantity, quality, and spacing: Density-regulation mechanisms in bark beetle *Ips typographus*. Journal of Chemical Ecology, 13, 1503–1523. DOI: not provided in supplied text / verify.

Schlyter, F., Birgersson, G., Byers, J. A., & Bakke, A. (1992). The aggregation pheromone of *Ips duplicatus* and its role in competitive interactions with *I. typographus*(Coleoptera: Scolytidae). Chemoecology, 3(3), 103-112.https://doi.org/10.1007/BF01370137

Schlyter, F., Jakuš, R., Han, F. Z., Ma, J. H., Kalinová, B., Mezei, P., ... & Zhang, Q. H. (2015). Reproductive isolation of Ips nitidus and I. shangrila in mountain forests of Western China: responses to chiral and achiral candidate pheromone

components. Journal of chemical ecology, 41(7), 678-688.https://doi.org/10.1007/s10886-015-0594-6

Schrey, S. D., &Tarkka, M. T. (2008). Friends and foes: streptomycetes as modulators of plant disease and symbiosis. Antonie Van Leeuwenhoek, 94(1), 11-19.https://doi.org/10.1007/s10482-008-9241-3

Schmidt, H. R., & Benton, R. (2020). Molecular mechanisms of olfactory detection in insects: beyond receptors. Open biology, 10(10).https://doi.org/10.1098/rsob.200252

Sousa, M., Birgersson, G., Karlsson Green, K., Pollet, M., & Becher, P. G. (2023). Odors attracting the long-legged predator Medeterasignaticornis Loew to *Ips typographus* L.-infested Norway spruce trees. Journal of Chemical Ecology, 49, 1–14. https://doi.org/10.1007/s10886-023-01405-6

Sousa, M., Andersson, A., Englund, J.-E., Flöhr, A., Pollet, M., Karlsson Green, K., Birgersson, G., & Becher, P. G. (2024). Behavioural 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. Annals of Forest Science, 81, 42. https://doi.org/10.1186/s13595-024-01261-8

Stadelmann, G., Bugmann, H., Meier, F., Wermelinger, B., & Bigler, C. (2013). Effects of salvage logging and sanitation felling on bark beetle (*Ips typographus* L.) infestations. Forest Ecology and Management, 305, 273-281.https://doi.org/10.1016/j.foreco.2013.06.003

Stříbrská, B., Hradecký, J., Čepl, J., Tomášková, I., Jakuš, R., Modlinger, R., & Jirošová, A. (2022). Forest margins provide favourable microclimatic niches to swarming bark beetles, but Norway spruce trees were not attacked by *Ips typographus* shortly after edge creation in a field experiment. Forest Ecology and Management, 506, 119950. https://doi.org/10.1016/j.foreco.2021.119950

Stříbrská, B., Hradecký, J., Čepl, J., Modlinger, R., Tomášková, I., & Jirošová, A. (2023). Physiological and biochemical indicators in Norway spruces freshly infested by *Ips typographus*: Potential for early detection methods. Frontiers in

Forests and Global Change, 6, 1197229. https://doi.org/10.3389/ffgc.2023.1197229

Sullivan, B. T., Berisford, C. W., & Dalusky, M. J. (1997). Field response of southern pine beetle parasitoids to some natural attractants. Journal of Chemical Ecology, 23, 837–856. https://doi.org/10.1023/B:JOEC.0000006415.17532.d0

Sun, X. L., Yang, Q. Y., Sweeney, J. D., & Gao, C. Q. (2006). A review: chemical ecology of *Ips typographus* (Coleoptera, Scolytidae). Journal of forestry research, 17(1), 65-70.https://doi.org/10.1007/s11676-006-0016-2

Sun, R., Hu, B., Nakamura, Y., Reichelt, M., Jiang, X., Luck, K., Gershenzon, J. (2025). Detoxification of conifer antimicrobial defenses promotes entomopathogenic fungus infection of bark beetles. Proceedings of the National Academy of Sciences, 123(1), e2525513122.https://doi.org/10.1073/pnas.2525513122

Šramel, N., Kavčič, A., Kolšek, M., & De Groot, M. (2021). Estimating the most effective and economical pheromone for monitoring the European spruce bark beetle. Journal of applied entomology, 145(4), 312-325. https://doi.org/10.1111/jen.12853

Symonds, M. R., & Elgar, M. A. (2004). The mode of pheromone evolution: evidence from bark beetles. Proceedings of the Royal Society of London. Series B: Biological Sciences, 271(1541), 839-846.https://doi.org/10.1098/rspb.2003.2647

Tanin, S. M., Kandasamy, D., & Krokene, P. (2021). Fungal interactions and host tree preferences in the spruce bark beetle *Ips typographus*. Frontiers in Microbiology, 12, 695167. https://doi.org/10.3389/fmicb.2021.695167

Tømmerås, B. Å. (1985). Specialization of the olfactory receptor cells in the bark beetle *Ips typographus* and its predator Thanasimusformicarius to bark beetle pheromones and host tree volatiles. Journal of Comparative Physiology A, 157(3), 335-341. https://doi.org/10.1007/BF00618123

Unelius, C. R., Schiebe, C., Bohman, B., Andersson, M. N., & Schlyter, F. (2014). Non-host volatile blend optimization for forest protection against the European

spruce bark beetle, Ipstypographus. PLoS One, 9(1), e85381.https://doi.org/10.1371/journal.pone.0085381

UNECE - Region Forests and Forest Sector (2023). The Geneva-based UNECE/FAO Forestry and Timber. Available in: https://forest-data.unece.org/Countries/CZ#17

Vega, F. E., and Harry K. Kaya. Insect pathology. In:Fungal Entomopathogens Academic press, 2012. https://doi.org/10.1016/B978-0-12-384984-7.00006-3

Vega, F. E., & Hofstetter, R. W. (Eds.). (2015). Bark beetles: biology and ecology of native and invasive species. Academic Press.ISBN: 978-0-12-417156-5. https://doi.org/10.1016/C2013-0-04752-4

Veselská, T., Švec, K., Kostovčík, M., Peral-Aranega, E., Garcia-Fraile, P., Křížková, B., ... & Kolařík, M. (2023). Proportions of taxa belonging to the gut core microbiome change throughout the life cycle and season of the bark beetle *Ips typographus*. FEMS Microbiology Ecology, 99(8), fiad072.https://doi.org/10.1093/femsec/fiad072

Vité, J. P., Bakke, A., & Renwick, J. A. A. (1972). Pheromones in Ips (Coleoptera: Scolytidae): occurrence and production1. The Canadian Entomologist, 104(12), 1967-1975.https://doi.org/10.4039/Ent1041967-12

Vité, J. P., Hedden, R., & Mori, K. (1976). Ips grandicollis: field response to the optically pure pheromone.https://doi.org/10.1007/BF00768689.

Vité, J. P., Ohloff, G., & Billings, R. F. (1978). Pheromonal chirality and integrity of aggregation response in southern species of the bark beetle Ips sp. Nature, 272(5656), 817-818.https://doi.org/10.1038/272817a0

Wadke, N., Kandasamy, D., Vogel, H., Lah, L., Wingfield, B. D., Paetz, C., ... & Hammerbacher, A. (2016). The bark-beetle-associated fungus, Endoconidiophorapolonica, utilizes the phenolic defense compounds of its host as a carbon source. Plant physiology, 171(2), 914-931.https://doi.org/10.1104/pp.15.01916

Wang, Z., Liang, L., Wang, H., Decock, C., & Lu, Q. (2024). Ophiostomatoid fungi associated with Ips bark beetles in China. Fungal Diversity, 129(1), 283-364.https://doi.org/10.1007/s13225-024-00546-7

Wegensteiner, R., Wermelinger, B., & Herrmann, M. (2015). Natural enemies of bark beetles: Predators, parasitoids, pathogens, and nematodes. In F. E. Vega & R. W. Hofstetter (Eds.), Bark beetles: Biology and ecology of native and invasive species (pp. 247–304). Academic Press. https://doi.org/10.1016/B978-0-12-417156-5.00007-1

Wermelinger, B., & Seifert, M. (1998). Analysis of the temperature dependent development of the spruce bark beetle *Ips typographus* (L)(Col., Scolytidae). Journal of Applied Entomology, 122(1-5), 185-191.https://doi.org/10.1111/j.1439-0418.1998.tb01482.x

Wermelinger, B., & Seifert, M. (1999). Temperature-dependent reproduction of the spruce bark beetle *Ips typographus*, and analysis of the potential population growth. Ecological Entomology, 24(1), 103-110.https://doi.org/10.1046/j.1365-2311.1999.00175.x

Wermelinger, B. (2002). Development and distribution of predators and parasitoids during two consecutive years of an *Ips typographus* (Col., Scolytidae) infestation. Journal of Applied Entomology. https://doi.org/10.1046/j.1439-0418.2002.00707.x

Wermelinger, B. (2004). Ecology and management of the spruce bark beetle *Ips typographus*: A review of recent research. Forest Ecology and Management, 202(1–3), 67–82. https://doi.org/10.1016/j.foreco.2004.07.018

Wermelinger, B., Epper, C., Kenis, M., Ghosh, S., &Holdenrieder, O. (2012). Emergence patterns of univoltine and bivoltine *Ips typographus* (L.) populations and associated natural enemies. Journal of Applied Entomology, 136(3), 212-224.

Wegensteiner, R., Wermelinger, B., & Herrmann, M. (2015). Natural enemies of bark beetles: Predators, parasitoids, pathogens, and nematodes. In F. E. Vega & R. W. Hofstetter (Eds.), Bark beetles: Biology and ecology of native and invasive species. Elsevier. https://doi.org/10.1016/B978-0-12-417156-5.00007-1

Weslien, J., &Regnander, J. (1992). The influence of natural enemies on brood production in *Ips typographus* (Col. Scolytidae), with special reference to egg-laying and predation by Thanasimusformicarius (Col. Cleridae). Entomophaga. https://doi.org/10.1007/BF02372435

Weslien, J., Annila, E., Bakke, A., Bejer, B., Eidmann, H. H., Narvestad, K., ... & Ravn, H. P. (1989). Estimating risks for spruce bark beetle (*Ips typographus* (L.)) damage using pheromone-baited traps and trees. Scandinavian Journal of Forest Research, 4(1-4), 87-98.https://doi.org/10.1080/02827588909382549

Welch, K. D., & Harwood, J. D. (2014). Temporal dynamics of natural enemy–pest interactions in a changing environment. Biological control, 75, 18-27.https://doi.org/10.1016/j.biocontrol.2014.01.004

Werner, R. A. (1995). Toxicity and repellency of 4–allylanisole and monoterpenes from white spruce and tamarack to the spruce beetle and eastern larch beetle (Coleoptera: Scolytidae). Environmental Entomology, 24(2), 372-379. https://doi.org/10.1093/ee/24.2.372

Wittstock, U., & Gershenzon, J. (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. Current opinion in plant biology, 5(4), 300-307.https://doi.org/10.1016/S1369-5266(02)00264-9

Wielkopolan, B., Jakubowska, M., &Obrępalska-Stęplowska, A. (2021). Beetles as plant pathogen vectors. Frontiers in Plant Science, 12, 748093.https://doi.org/10.3389/fpls.2021.748093

Wolff, G. H., and Riffell, J. A. (2018). Olfaction, experience and neural mechanisms underlying mosquito host preference. Journal of Experimental Biology, 221(4), jeb157131. https://doi.org/10.1242/jeb.157131

Wood, D. L. (1982). The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Annual Review of Entomology, 27, 411–446. https://doi.org/10.1146/annurev.en.27.010182.002211

Yan, H., Jafari, S., Pask, G., Zhou, X., Reinberg, D., &Desplan, C. (2020). Evolution, developmental expression and function of odorant receptors in insects. Journal of Experimental Biology, 223(Suppl_1), jeb208215.https://doi.org/10.1242/jeb.208215

Zaman, R., Jain, A., Hammerbacher, A., Gershenzon, J., & Kandasamy, D. (2025). Both synergism and interaction diversity explain the mixtures of defensive

monoterpenes in spruce oleoresin. Functional Ecology, 39(9), 2539-2551. https://doi.org/10.1111/1365-2435.70077

Zúbrik, M., Kunca, A., Kulfan, J., Rell, S., Nikolov, C., Galko, J., ... & Holuša, J. (2021). Occurrence of gypsy moth (*Lymantria dispar* L.) in the Slovak Republic and its outbreaks during 1945–2020. Central European Forestry Journal, 67(2), 55-71. 10.2478/forj-2021-000710.2478/forj-2021-0007

Zhang, Q. H., Tolasch, T., Schlyter, F., & Francke, W. (2002). Enantiospecific antennal response of bark beetles to spiroacetal (E)-conophthorin. Journal of Chemical Ecology, 28(9), 1839-1852.https://doi.org/10.1023/A:1020569303433

Zhang, Q.-H., & Schlyter, F. (2003). Redundancy, synergism, and active inhibitory range of non-host volatiles in reducing pheromone attraction in European spruce bark beetle *Ips typographus*. Oikos, 101, 299–310. https://doi.org/10.1034/j.1600-0706.2003.11168.x

Zhang, Q. H., & Schlyter, F. (2004). Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles. Agricultural and forest entomology, 6(1), 1-20.https://doi.org/10.1111/j.1461-9555.2004.00202.x

Zhang, Q. H., Ma, J. H., Zhao, F. Y., Song, L. W., Sun, J. H., & Cognato, A. I. (2011). Aggregation pheromone of the Oriental spruce engraver Pseudipsorientalis. Agricultural and Forest Entomology, 13(1), 67-75.https://doi.org/10.1111/j.1461-9563.2010.00500.x

Zhang, Q. H., Schlyter, F., Chen, G., & Wang, Y. (2007). Electrophysiological and behavioural responses of Ips subelongatus to semiochemicals from its hosts, non-hosts, and conspecifics in China. Journal of chemical ecology, 33(2), 391-404.https://doi.org/10.1007/s10886-006-9231-8

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

Appendix

Publication I

Stříbrská, B**., Moliterno, A. A. C**., Hüttnerová, T., Leiner, M., Surový, P., & Jirošová, A. (2024). Pilot study of the 3D spatial distribution of *α*-pinene emitted by Norway spruce, *Picea abies* (L.) Karst., recently infested by *Ips typographus* (L. 1758) (Coleoptera: Scolytinae). Forests, 15(1), 10. https://doi.org/10.3390/f15010010

Publication II

Moliterno, A. A. C., Jakuš, R., Modlinger, R., Unelius, C. R., Schlyter, F., & Jirošová, A. (2023). Field effects of oxygenated monoterpenes and estragole combined with pheromone on the attraction of *Ips typographus* and its natural enemies. Frontiers in Forests and Global Change, 6, 1292581. https://doi.org/10.3389/ffgc.2023.1292581

Publication III

Moliterno, A. A. C., Shewale, M. K., Basile, S., Synek, J., & Jirošová, A. (2025). Size-dependent behavioural and antennal responses to doses of (+)-isopinocamphone and 1,8-cineole mixed with pheromone: A potential host-selection strategy in female *Ips typographus* L. Annals of Forest Science, 82(1), 34. https://doi.org/10.1186/s13595-025-01297-4