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# Using ethanol and other lures to monitor invasive ambrosia beetles in endemic populations: case study from the Czech Republic

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Bark and ambrosia beetles pose significant threats to the stability of forest stands worldwide, making their control crucial. Among these pests, *Gnathotrichus materiarius*, a polyphagous invasive ambrosia beetle living on conifers, has successfully established itself in Europe. Early identification of these pests plays a fundamental role in designing effective pest control strategies. The work aimed to assess the efficacy of different lures in Ecotrap® for capturing of invasive ambrosia bark beetles. The lures tested included Wood Stainers Lure (containing the potential pheromone sulcatol for capturing *Gnathotrichus materiarius* adults),  $\alpha$ -pinene, ethanol UHR, and Cembräwit. The objective was to determine the most suitable lure for use in traps. In four locations in western Bohemia, a total of 7,410 individuals from 46 species of ambrosia and bark beetles were captured. The abundance of invasive ambrosia beetles (*Gnathotrichus materiarius*, *Cyclorhipidion bodoanum*, *Xyleborinus attenuatus*, and *Xylosandrus germanus*) primarily depended on the day of the season and secondarily on the lure used. Although their population density was low, more beetles were caught using ethanol as the lure. Notably, these invasive ambrosia beetles accounted for less than 3 % of the total number of ambrosia and bark beetles detected (187 individuals). Ethanol was found to be a universal lure for attracting ambrosia beetles, with the majority of Scolytinae species being captured in traps baited with ethanol.

## KEYWORDS

$\alpha$ -pinene, Cembräwit, *Cyclorhipidion bodoanum*, *Gnathotrichus materiarius*, sulcatol, *Xylosandrus germanus*, *Xyleborinus attenuatus*

## 1. Introduction

Bark and ambrosia beetles are among the most serious pests that threaten the stability of forest stands worldwide (Hulcr and Dunn, 2011; Hlásny et al., 2021). While bark beetles feed on the phloem, interrupting the transport of substances produced by photosynthesis and utilizing transferred fungi to overcome the defensive reactions of the host tree, ambrosia beetles feed on fungi carried in their mycangia and actively cultivate them on gallery walls within the wood (Hulcr et al., 2007). Invasive bark and ambrosia beetles pose threats not only to native biodiversity and functional ecosystems but also to the economic productivity of forest management (Brockhoff et al., 2006a; Aukema et al., 2011; Gohli et al., 2016). Early identification of pests is a fundamental step in invasive bark and ambrosia beetle control, as it aids in the design of pest management strategies (Douglas et al., 2009).

Implementing integrated control measures proves more cost-effective than the loss of timber (Franjević et al., 2016). For example, failure to monitor the invasive ambrosia beetle in *Pinus taeda* L. forests in the southern USA has resulted in an estimated economic loss amounting to several hundred dollars per hectare for landowners (Susaeta et al., 2016). At the same time, lures require smaller financial expenditure than the human labor associated with the control of traps (Šramel et al., 2021).

Bark and ambrosia beetles use a complex chemical communication system (utilizing pheromones, allomones, kairomones, synomones) to locate and infest new host trees for feeding, mating, and reproduction (Wood, 1982; Hulcr et al., 2007; Seybold et al., 2018). Trees play a crucial role as producers of kairomones. When trees experience stress due to factors like drought, frost, floods, fire, or human-induced damage, they release ethanol, which is the most important volatile compound attractive to ambrosia beetles (Kühnholz et al., 2001; Kelsey and Joseph, 2003; Ranger et al., 2013, 2019, 2021). Different combinations of volatile compounds have been employed to monitor and detect bark and ambrosia beetles at an early stage. One of the most used approaches involves the simultaneous use of ethanol and  $\alpha$ -pinene (Borden et al., 1980; Schroeder and Lindelöw, 1989; Miller and Rabaglia, 2009; Burbano et al., 2012; Flaherty et al., 2019).

In Europe, most invasive ambrosia beetles originate from temperate or subtropical regions. When these invasive beetles colonize new territories with suitable conditions, their population numbers can increase dramatically over time (Galko et al., 2014; Boland, 2016). Conversely, under unsuitable conditions, the abundance of ambrosia beetles remains low, or the population will even disappear (Fiala et al., 2020). *Gnathotrichus materiarius* Fitch, 1858 a polyphagous invasive ambrosia beetle that primarily infests conifers, has successfully spread throughout Europe (Kamp, 1970). Despite its presence in European forests for nearly a century, no significant damage has been reported from *G. materiarius* infestations (Mazur et al., 2018). Although we can lure this ambrosia beetle to host tree logs (Fiala et al. in prep.), it is often caught in traps baited with lures designed for capturing bark beetles of the *Ips* genus (Valkama et al., 1997; Fiala, 2019). Commonly used lures for this purpose include ID Ecolure®, IT Ecolure®, Cembräwit®, Amitinuswit®, Hostowit®, and ethanol combined with other lures (Schneider, 1985; Knížek, 2009; Mazur et al., 2018). It is worth noting that sulcatol, a potential aggregation pheromone, has also been identified (Flechtmann and Berisford, 2003) and for other member of Corthylini ambrosia beetles, *Monarthrum mali* Fitch, 1856 (Miller and Crowe, 2020). All mentioned lures are alcohol-based, aligning with the use of ethanol for capturing ambrosia beetles (Lingren and Fraser, 1994; Rassati et al., 2015; Rabaglia et al., 2019).

We aimed to: (i) Analyze the effectiveness of various lure types on capturing invasive ambrosia beetles in endemic populations in central Europe; (ii) Analyze the impact of various lure types on species richness and abundance of both bark and ambrosia beetles, identifying their distinct responses to lure treatments; (iii) Examine the proportions of invasive ambrosia beetles and native coniferous bark beetles in relation to lure attractiveness, emphasizing the lure preferences of each group; (iv) Assess the potential interaction effects between season and lure type, elucidating how these factors jointly influence beetle behavior and their distribution across the study period.

This work aimed to compare the attractiveness of different lures and determine which one is most effective in attracting *G. materiarius*.

The lures tested included Wood Stainers Lure, containing the pheromone sulcatol (Flechtmann and Berisford, 2003);  $\alpha$ -pinene due to the preference of coniferous bark beetles (Schroeder and Lindelöw, 1989); ethanol as *G. materiarius* is an ambrosia beetle known to respond to ethanol (Kelsey and Joseph, 2003), and Cembräwit since *G. materiarius* has shown a positive response also to this lure (Schneider, 1985). We predicted greater catches of invasive ambrosia beetles, coniferous bark beetles, and *Ips* spp., in traps baited, respectively, with ethanol,  $\alpha$ -pinene, and the Cembräwit lure.

## 2. Materials and methods

### 2.1. Study localities

The experiments were conducted in four localities in western Bohemia (Czech Republic), in 2022. The invasive ambrosia beetle species, *G. materiarius*, *Xylosandrus germanus*, *Cyclorhipidion bodoanum* Reitter, 1913, and *Xyleborinus attenuatus* Blandford, 1894, were found to occur in the studied areas. All the localities are coniferous forests, namely Úbočí (50.0259°N, 12.5859°E, 750 m asl), Kladská (50.0116°N, 12.6746°E, 865 m asl), Žihle (50.0391°N, 13.3520°E, 510 m asl), and Kdyně (GPS 49.4024°N, 13.0995°E, 600 m asl) (Figure 1).

### 2.2. Trapping

In 2022, four lures were compared for their ability to attract and trap *G. materiarius*: Cembräwit (as the control, Witasek GmbH, Austria),  $\alpha$ -pinene, ethanol UHR (all from Synergy Semiochemicals Corp., United States), and Wood Stainers Lure (a mixture of  $\alpha$ -pinene, ethanol, and sulcatol) (abbr. as WSL) (Alpha Scents, Inc., United States) (Table 1). All four lures were deployed in traps across all four localities. Each locality had 20 Ecotrap® traps (4 treatments  $\times$  5 replicates per treatment) (Fytofarm spol. s r.o., Slovakia) arranged in a linear transect at 10-meter intervals. This interval is sufficient because the effectiveness of traps on flying bark beetles can be calculated in units of meters (Duelli et al., 1997). The traps were placed 130 cm above the ground using wooden stakes along an east-west transect, with one lure assigned per trap. Randomly placed in the trap row, there were five replicates per lure treatment per locality.

The traps, baited with the lures, were deployed at the end of April, and checked every 14 days until mid-August. The collected beetles were frozen and subsequently identified by the first author according to Pfeffer (1989). Even if the beetles exceptionally had been damaged by predators, it was possible to study the remains and determine the number of specimens and species.

### 2.3. Statistical analysis

The data were processed using R 4.2.2 (R Core Team, 2022). Negative Binomial Zero-Inflated Mixed Models from the NBZIMM library (Yi, 2020) were fitted to analyze the richness and abundance of bark and ambrosia beetles. The response variables included the abundance of all beetles, the richness of all beetles, the overall abundance of invasive ambrosia beetles (*C. bodoanum*, *G. materiarius*,

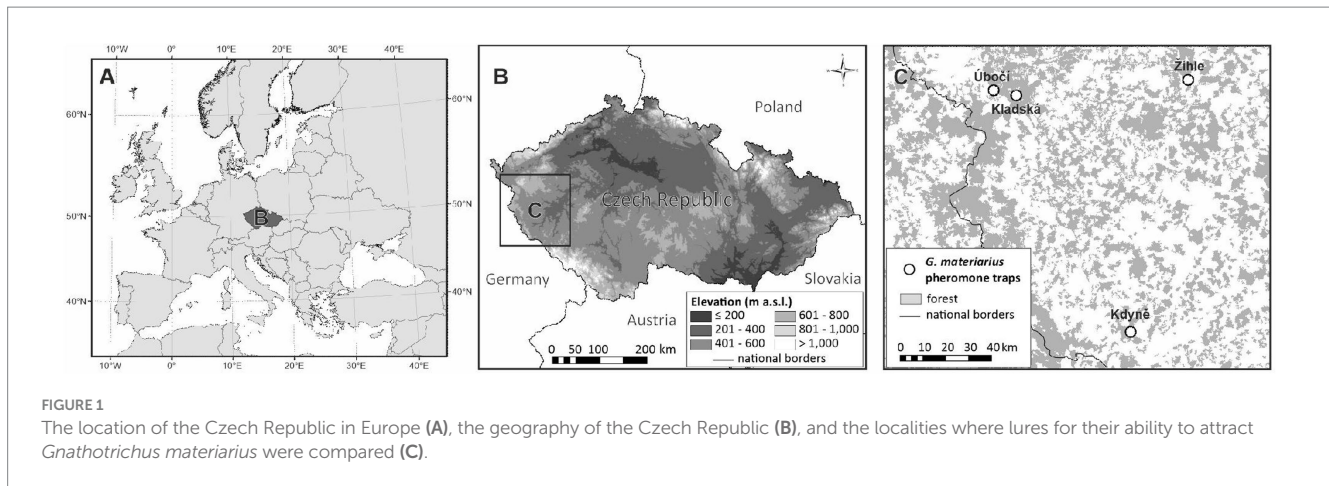


FIGURE 1 The location of the Czech Republic in Europe (A), the geography of the Czech Republic (B), and the localities where lures for their ability to attract *Gnathotrichus materiarius* were compared (C).

TABLE 1 Descriptions of the lures used for catching *Gnathotrichus materiarius*.

Lure	Abbreviations	Release rate (g/day)/type of package	Compounds	CAS number
Alpha-pinene	$\alpha$ -pinene	0.070/bubble cap		80-56-8
Cembräwit	Cembräwit	0.060/pouch	Ipsenol, ipsdienol, methyl butenol and amitinol	
Wood Stainers lure	WSL	0.362/bubble cap	Sulcatol, alpha-pinene, ethanol; 1:1:1	Sulcatol: 1569-60-4 alpha-pinene: 80-56-8 ethanol: 64-17-5
Ethanol UHR	Ethanol	0.300/pouch		64-17-5

*X. germanus*, and *X. attenuatus*), their richness, and their individual abundances (except for *X. attenuatus*, where the model was omitted due to limited data). As potential explanatory variables, we considered the type of lure, the day of the season (dos), the quadratic polynomial of the dos, and the interaction between dos and the type of lure as this analysis focused on investigating the season-part-dependent effect of individual lures. The model incorporated sampling location, individual plots nested within the sampling location, and individual traps nested in plots as random terms. These components simultaneously defined the autocorrelation structure as “ar1” in the model.

For the second part of the analysis, Generalized Linear Mixed Models with binomial distribution from the lme4 library (Bates et al., 2015) were employed. Since the focus shifted away from the season, the catches from individual traps were aggregated across the season. The explanatory variable considered was the type of substance, while the random terms included sampling location and individual plots nested within the sampling location. The response variables were the proportions of individuals of invasive ambrosia beetles in all ambrosia beetles, the genus *Ips*, and native coniferous bark beetles (excluding *Ips*) in all individuals. The resulting models were compared with the respective null models to assess their fit. To monitor potential autocorrelation, auto- and cross-covariance and -correlation function estimation was performed on the residuals of the model. Given the interdependence of proportions, the resulting *p*-values were adjusted based on the false discovery rate. Potential convergence issues in the algorithm were addressed by setting the maximum number of iterations for the model to  $10^5$ , establishing a tolPwrss

tolerance level of  $10^{-3}$  for declaring convergence in the penalized iteratively weighted residual sum-of-squares, and utilizing the Gauss-Hermite algorithm. Furthermore, in the model examining the ratio of individuals of invasive ambrosia beetles to all individuals, the sampling location Kladská and  $\alpha$ -pinene lure were excluded from the analysis. This decision was made due to the absence of recorded invasive ambrosia beetles. In the analyses, WSL was chosen as the control group due to the absence of invasive ambrosia beetles on  $\alpha$ -pinene and their limited abundance on Cembräwit.

Partial Canonical Correspondence Analysis (p-CCA) from Canoco 5.01 (ter Braak and Šmilauer, 2012) was utilized to investigate whether the dos and lure had a significant impact on the composition of beetle communities, with sampling locations included as a covariate. Before the analysis, rare species were down-weighted, and the results were assessed using the Monte-Carlo permutation test with 999 permutations. To identify indicator species for each lure, the IndVal method was employed, which considers the frequency and relative abundance of the beetles (Dufrière and Legendre, 1997). This was followed by multilevel pattern analysis using “indicpecies” (De Cáceres and Legendre, 2009) and “labdsv” (Roberts, 2019) libraries. Most of the plots were generated using the sciplot library (Morales, 2020).

### 3. Results

In total, 46 species of bark and ambrosia beetles were caught amounting to a total of 7,410 individuals (Supplementary Table S1).

### 3.1. Invasive ambrosia beetles

The numbers of *G. materiarius* captured were primarily dependent on the day of the season ( $df_{num}=1$ ,  $df_{den}=556$ ,  $F=119.65$ ,  $p<0.001$ ), with higher numbers observed at the beginning of the season. The lure type also had an effect ( $df_{num}=3$ ,  $df_{den}=57$ ,  $F=4.95$ ,  $p=0.004$ ), where most beetles were caught using ethanol (Figure 2A). The interaction between the day of the season and the lure was not found to be significant ( $df_{num}=3$ ,  $df_{den}=556$ ,  $F=1.69$ ,  $p=0.168$ ). When comparing the capture of *G. materiarius* in WSL-baited traps with other lure treatments, no beetles were captured with  $\alpha$ -pinene, fewer beetles were captured with Cembräwit, and more beetles were captured with ethanol.

The numbers of *C. bodoanum* captured were significantly influenced by the day of the season ( $df_{num}=1$ ,  $df_{den}=556$ ,  $F=405.82$ ,  $p<0.001$ ) with higher numbers observed at the beginning of the season. The lure type, on the other hand, had an insignificant effect ( $df_{num}=3$ ,  $df_{den}=57$ ,  $F=0.80$ ,  $p=0.497$ ). However, there was a strong interaction between the day of the season and the type of lure ( $df_{num}=3$ ,  $df_{den}=556$ ,  $F=35.80$ ,  $p<0.001$ ). The highest number of beetles was found in traps baited with ethanol at the beginning of the season (Figure 2B).

The numbers of *X. germanus* were significantly influenced by the day of the season ( $df_{num}=1$ ,  $df_{den}=556$ ,  $F=633.56$ ,  $p<0.001$ ) with higher numbers observed at the beginning of the season. The lure type, however, had an insignificant effect ( $df_{num}=3$ ,  $df_{den}=57$ ,  $F=0.81$ ,  $p=0.491$ ). Nevertheless, there was a strong interaction between the day of the season and the type of lure ( $df_{num}=3$ ,  $df_{den}=556$ ,  $F=8.00$ ,  $p<0.001$ ). When comparing the catch with WSL-baited traps, no beetles were found on  $\alpha$ -pinene or Cembräwit, while a higher number of beetles were found on ethanol at the beginning of the season (Figure 2C).

### 3.2. Richness and abundance of bark and ambrosia beetles

The average species richness of ambrosia beetles per trap depended on the day of the season ( $df_{num}=2$ ,  $df_{den}=552$ ,  $F=115.26$ ,  $p<0.001$ ) with higher richness at the beginning of the season around the end of the May, and on the type of lure ( $df_{num}=3$ ,  $df_{den}=57$ ,  $F=39.96$ ,  $p<0.001$ ), the interaction between them was not significant ( $df_{num}=3$ ,  $df_{den}=552$ ,  $F=1.36$ ,  $p=0.228$ , Figure 3A). In comparison to WSL, lower species richness was captured on  $\alpha$ -pinene and Cembräwit, but higher species richness was captured on ethanol UHR.

The average abundance of ambrosia beetles per trap depended on the day of the season ( $df_{num}=2$ ,  $df_{den}=552$ ,  $F=274.99$ ,  $p<0.001$ ) with higher abundance at the beginning of the season around the end of the May, and on the type of lure ( $df_{num}=3$ ,  $df_{den}=57$ ,  $F=46.98$ ,  $p<0.001$ ) with their interaction ( $df_{num}=6$ ,  $df_{den}=552$ ,  $F=2.20$ ,  $p=0.041$ , Figure 3B). In comparison to WSL, lower abundance was captured on  $\alpha$ -pinene and Cembräwit, and similar abundance was captured on ethanol UHR with except of period from end of May till end of June, where ethanol UHR was most effective lure.

The average species richness of bark beetles per trap depended on the day of the season ( $df_{num}=2$ ,  $df_{den}=552$ ,  $F=156.16$ ,  $p<0.001$ ) with higher richness at the beginning of the season around the end of the May, and tended to depend on the type of lure ( $df_{num}=3$ ,  $df_{den}=57$ ,  $F=2.76$ ,  $p=0.050$ ), the interaction between them tended to be significant ( $df_{num}=3$ ,  $df_{den}=552$ ,  $F=1.87$ ,  $p=0.084$ , Figure 3C). WSL was the most effective lure at the beginning of the season, later in the season, its efficacy became comparable to other compounds, leading to a diminishing distinction in attractant preferences among bark beetles.

The average abundance of bark beetles per trap depended on the day of the season ( $df_{num}=2$ ,  $df_{den}=552$ ,  $F=187.65$ ,  $p<0.001$ ) with higher abundance at the beginning of the season around the end of

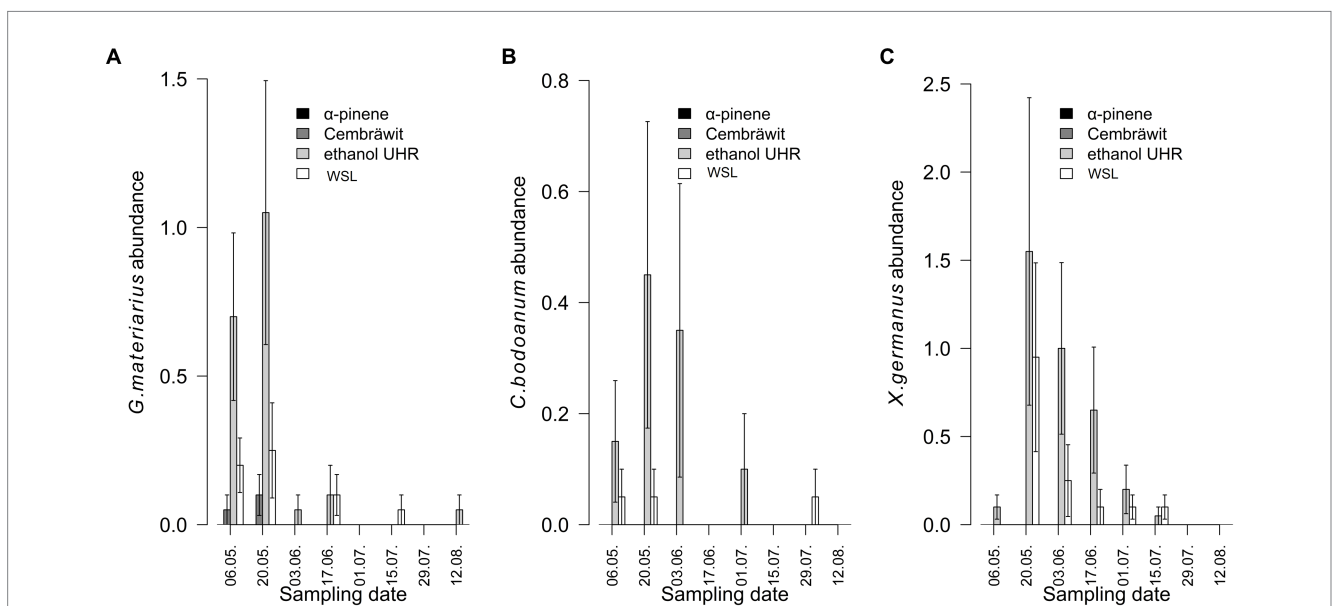


FIGURE 2

The numbers of ambrosia beetles caught per trap, including *Gnathotrichus materiarius* (A), *Cyclorhipidion bodoanum* (B), and *Xylosandrus germanus* (C), varied depending on the type of lure and the date of checking in year 2022 (mean  $\pm$  SE) (WSL, Wood Stainers lure).

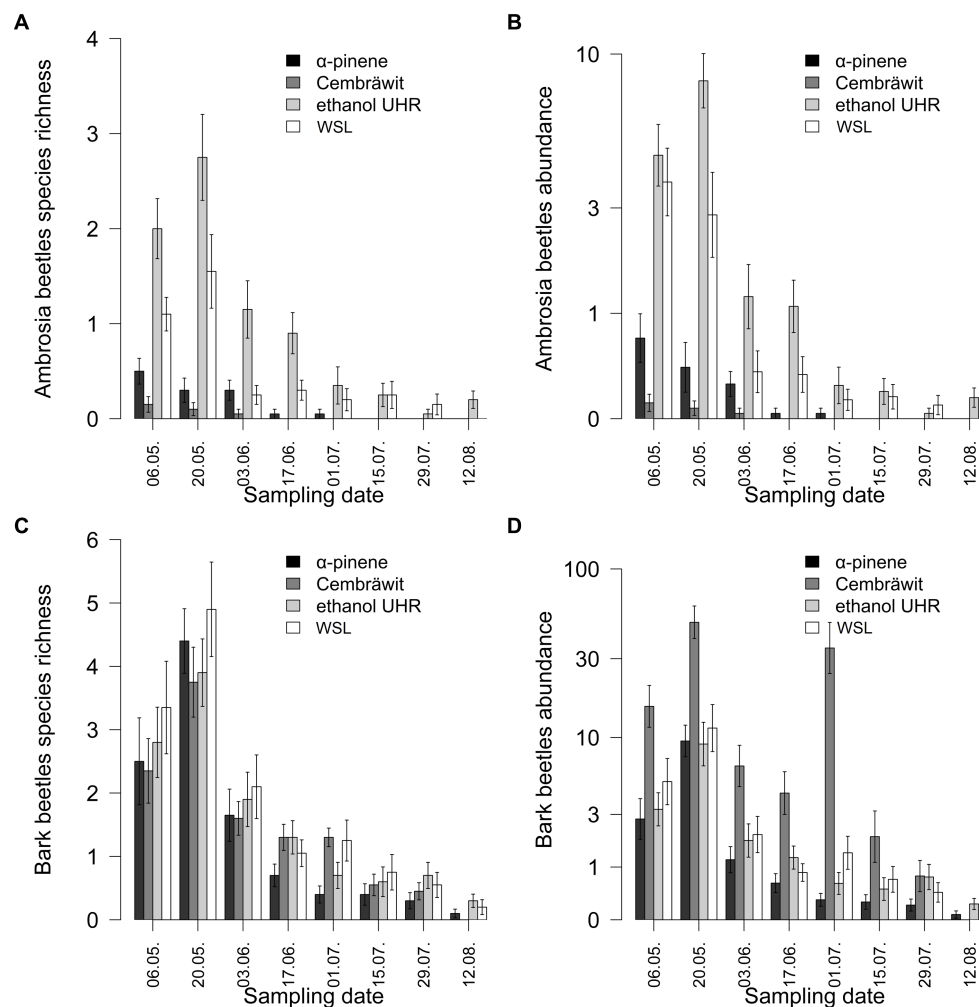


FIGURE 3

The richness (A,C) and abundance (B,D) of ambrosia and bark beetles varied depending on the type of lure and the date of checking in year 2022 (mean  $\pm$  SE) (WSL, Wood Stainers lure).

the May, and on the type of lure ( $df_{\text{num}}=3$ ,  $df_{\text{den}}=57$ ,  $F=52.86$ ,  $p<0.001$ ) with their interaction ( $df_{\text{num}}=6$ ,  $df_{\text{den}}=552$ ,  $F=12.55$ ,  $p<0.001$ , Figure 3D). In comparison to WSL, lower abundance was captured on  $\alpha$ -pinene, higher abundance was captured on Cembräwit, and similar abundance was captured on ethanol UHR. Cembräwit had a different progression of the season than the other types of lure—a second peak during the season and a much faster decline after that.

### 3.3. Richness and abundance of invasive species and all bark and ambrosia beetles

The average species richness of bark and ambrosia beetles per trap was significantly influenced by the day of the season ( $df_{\text{num}}=2$ ,  $df_{\text{den}}=552$ ,  $F=195.32$ ,  $p<0.001$ ), with higher richness observed at the beginning of the season, particularly around the end of the May. Additionally, the type of lure also had a significant effect ( $df_{\text{num}}=3$ ,  $df_{\text{den}}=57$ ,  $F=15.30$ ,  $p<0.001$ ). However, the interaction between them was not found to be significant ( $df_{\text{num}}=3$ ,  $df_{\text{den}}=552$ ,  $F=1.72$ ,  $p=0.114$ , Figure 4A). When comparing the species richness captured in WSL-baited traps with

other lure treatments, lower species richness was observed on  $\alpha$ -pinene and Cembräwit, while higher species richness was observed on ethanol.

The average abundance of bark and ambrosia beetles per trap was significantly influenced by the day of the season ( $df_{\text{num}}=2$ ,  $df_{\text{den}}=552$ ,  $F=226.86$ ,  $p<0.001$ ), with higher abundance observed at the beginning of the season, particularly around the end of the May. The type of lure also had a significant effect ( $df_{\text{num}}=3$ ,  $df_{\text{den}}=57$ ,  $F=63.26$ ,  $p<0.001$ ), and there was a significant interaction between them ( $df_{\text{num}}=6$ ,  $df_{\text{den}}=552$ ,  $F=14.49$ ,  $p<0.001$ , Figure 4B). When comparing the abundance captured in WSL-baited traps with other lure treatments, lower abundance was observed on  $\alpha$ -pinene, higher abundance was observed on Cembräwit, and similar abundance was observed on ethanol. Cembräwit showed a different progression throughout the season compared to the other lure types, with a second peak during the season and a faster decline afterward.

The average species richness of invasive beetles per trap was significantly influenced by the day of the season ( $df_{\text{num}}=2$ ,  $df_{\text{den}}=552$ ,  $F=91.80$ ,  $p<0.001$ ), with higher richness observed at the beginning of the season, particularly around the end of the May. The type of lure also had a significant effect ( $df_{\text{num}}=3$ ,  $df_{\text{den}}=57$ ,  $F=2.99$ ,  $p=0.038$ , Figure 4C).

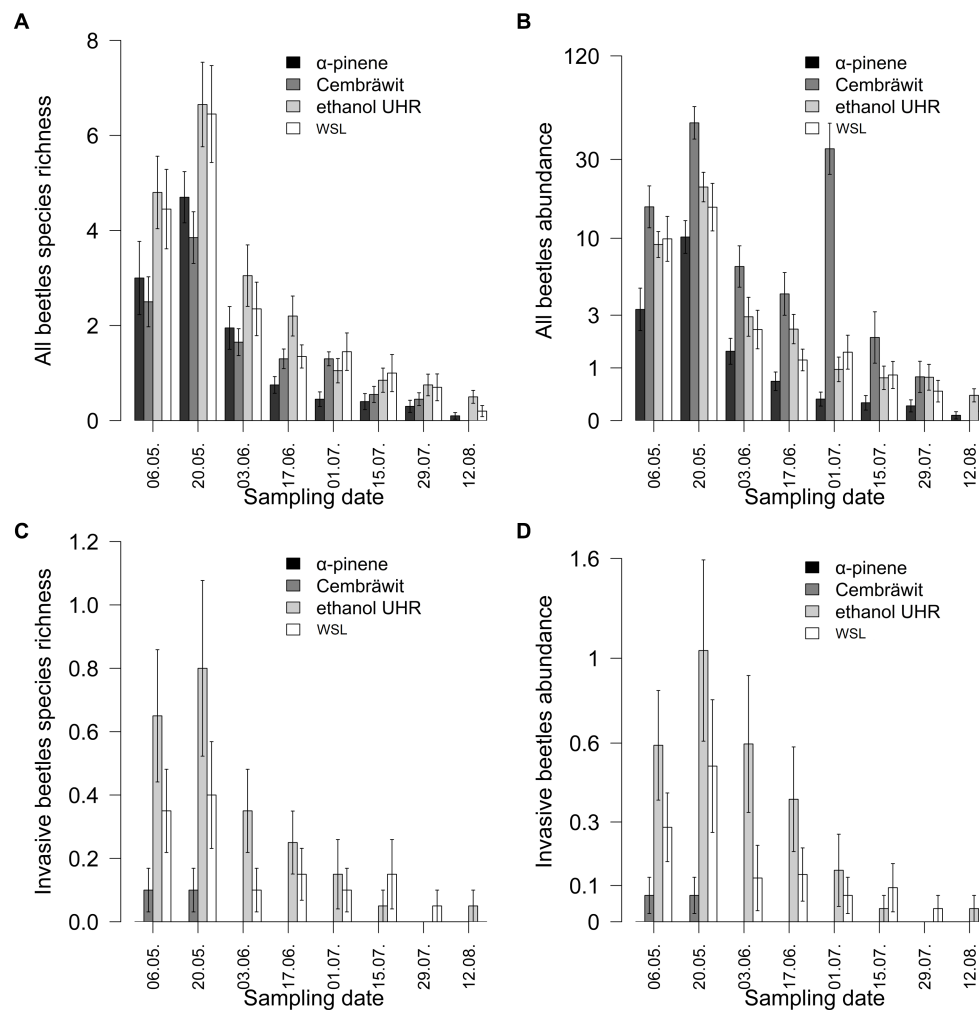


FIGURE 4

The richness (A,C) and abundance (B,D) of both invasive and all bark and ambrosia beetles varied depending on the type of lure and the date of checking in year 2022 (mean  $\pm$  SE) (WSL, Wood Stainers lure).

However, the interaction between them was not found to be significant ( $df_{num}=3$ ,  $df_{den}=552$ ,  $F=1.13$ ,  $p=0.339$ ). When comparing the species richness captured in WSL-baited traps with other lure treatments, no beetles were captured on  $\alpha$ -pinene, lower species richness was observed on Cembräwit, and higher species richness was observed on ethanol.

The average abundance of invasive beetles per trap is significantly influenced by the day of the season ( $df_{num}=2$ ,  $df_{den}=558$ ,  $F=98.05$ ,  $p<0.001$ ), with higher abundance observed at the beginning of the season, particularly around the end of the May. The type of lure also had a significant effect ( $df_{num}=3$ ,  $df_{den}=57$ ,  $F=5.89$ ,  $p=0.001$ , Figure 4D). When comparing the abundance captured in WSL-baited traps with other lure treatments, no beetles were captured on  $\alpha$ -pinene, lower abundance was observed on Cembräwit, and higher abundance was observed on ethanol.

### 3.4. Ratio of invasive beetles to all bark and ambrosia beetles

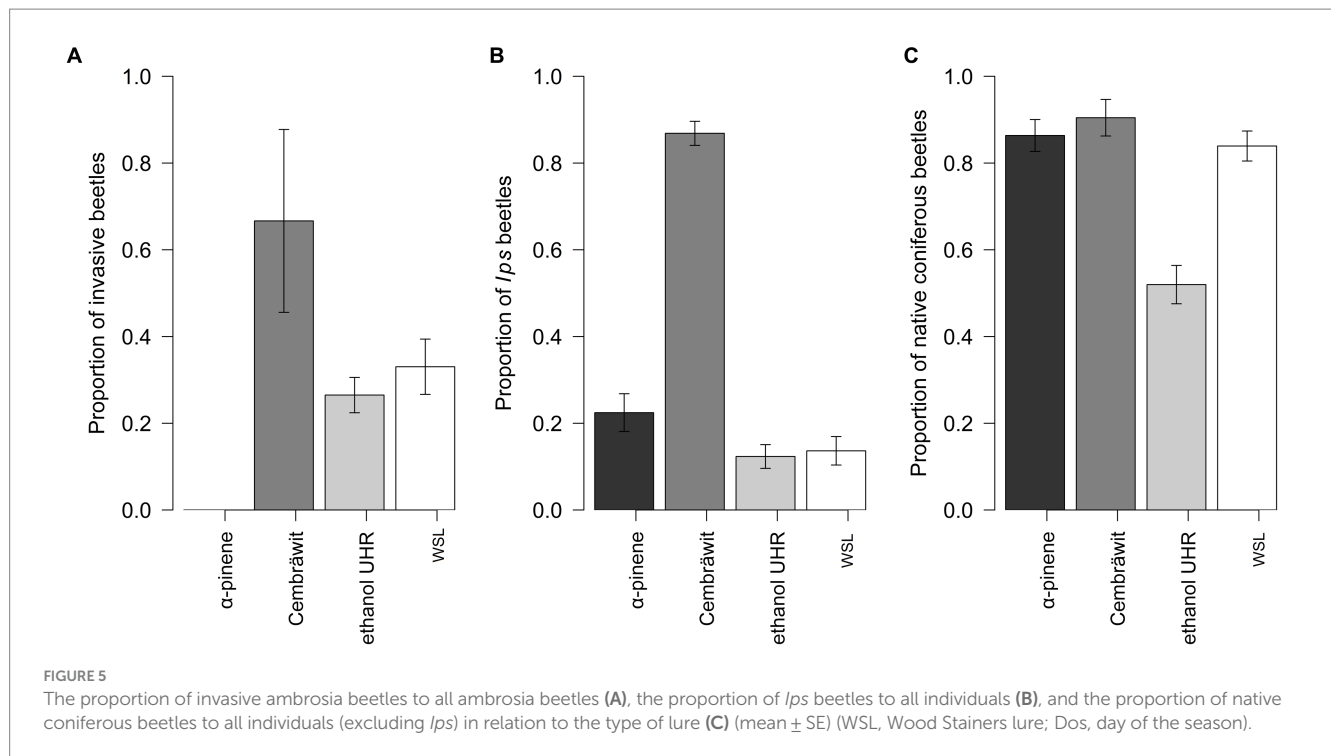
The proportion of invasive ambrosia beetles to all ambrosia beetles depended on the type of the lure ( $df=40$ ,  $\chi^2=10.52$ ,  $p=0.005$ , Figure 5A). In comparison to WSL, no beetles were captured on  $\alpha$ -pinene, the higher

proportion was captured on Cembräwit ( $z=2.96$ ,  $p=0.003$ ), and the lower proportion tended to be captured on ethanol UHR ( $z=1.69$ ,  $p=0.092$ ). The proportion of *Ips* beetles to all individuals was significantly influenced by the type of the lure ( $df=74$ ,  $\chi^2=5267.30$ ,  $p<0.001$ , Figure 5B). When comparing the proportion in WSL-baited traps with other lure treatments, there was a tendency for a higher proportion to be captured on  $\alpha$ -pinene ( $z=1.78$ ,  $p=0.075$ ), a higher proportion was captured on Cembräwit ( $z=37.51$ ,  $p<0.001$ ), and there was a tendency for a lower proportion to be captured on ethanol ( $z=-1.73$ ,  $p=0.083$ ).

The proportion of native coniferous beetles (excluding *Ips*) to all individuals was significantly influenced by the type of the lure ( $df=74$ ,  $\chi^2=33.65$ ,  $p<0.001$ , Figure 5C). When comparing the proportion in WSL-baited traps with other lure treatments, a similar proportion was captured on  $\alpha$ -pinene ( $z=0.19$ ,  $p=0.847$ ) and Cembräwit ( $z=0.10$ ,  $p=0.532$ ), while a lower proportion was captured on ethanol ( $z=-5.03$ ,  $p<0.001$ ).

### 3.5. Associated taxa

Of the 46 species of bark and ambrosia beetles identified, 16 were found to significantly respond to one or more lures. Table 2 presents



**TABLE 2** Species of bark and ambrosia beetles significantly associated with studied lures or their associations (WSL, Wood Stainers Lure).

Lure/lures associations	Species	Stat	<i>p</i> -value
Cembräwit	<i>Ips typographus</i>	0.668	0.001***
	<i>Ips cembrae</i>	0.379	0.001***
	<i>Pityokteines vorontzowi</i>	0.237	0.001***
Ethanol	<i>Anisandrus dispar</i>	0.487	0.001***
	<i>Cyclorhipidion bodoanum</i>	0.209	0.002**
$\alpha$ -Pinene and WSL	<i>Tomicus piniperda</i>	0.256	0.001***
	<i>Hylastes angustatus</i>	0.254	0.013*
	<i>Hylastes attenuatus</i>	0.241	0.015*
Ethanol and WSL	<i>Dryocoetes autographus</i>	0.503	0.001***
	<i>Xyleborinus saxesenii</i>	0.367	0.001***
	<i>Xylosandrus germanus</i>	0.301	0.001***
	<i>Gnathotrichus materiarius</i>	0.282	0.001***
	<i>Hylurgops palliatus</i>	0.244	0.001***
$\alpha$ -Pinene and Ethanol and WSL	<i>Trypodendron lineatum</i>	0.387	0.001***
Cembräwit and Ethanol and WSL	<i>Orthotomicus laricis</i>	0.25	0.029*

the species that were associated with some lure (i.e., they were caught on this lure at higher frequencies and abundances).

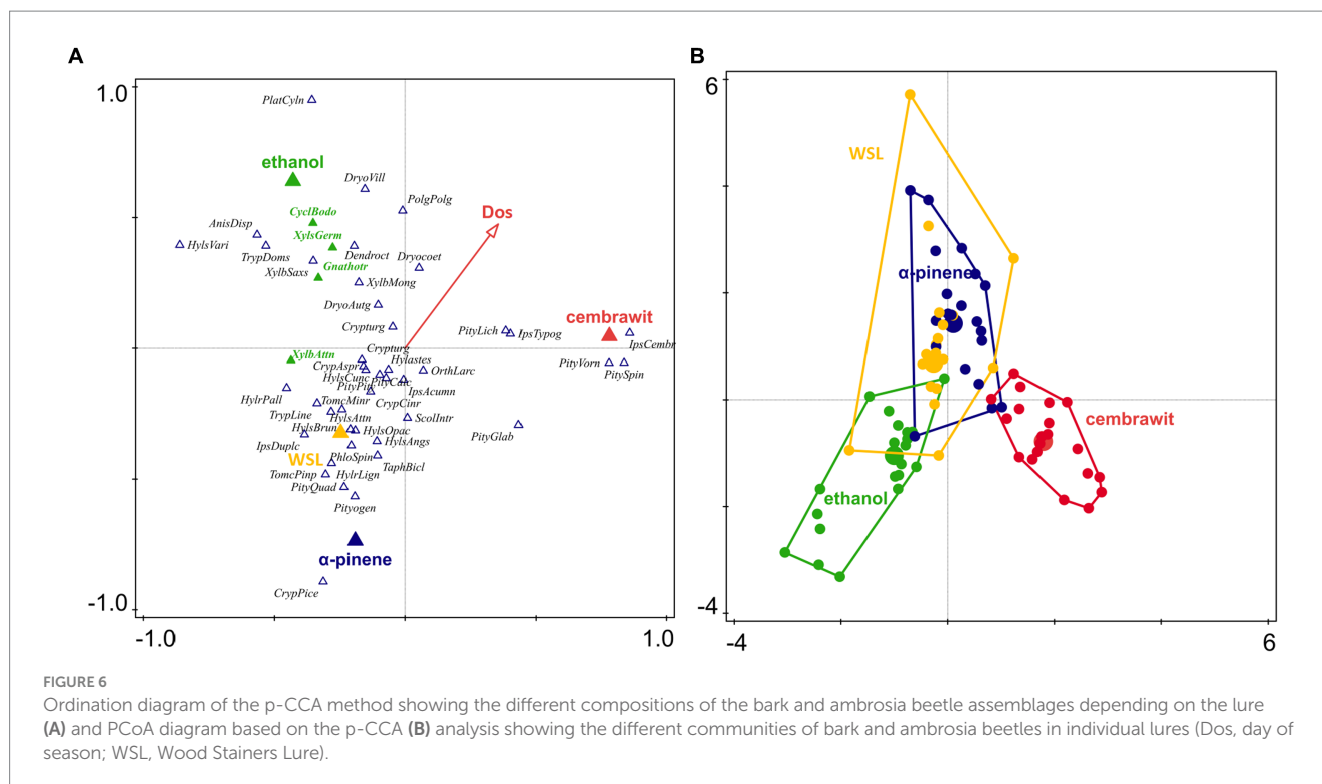
Furthermore, p-CCA analysis (after removing the effect of locality) revealed that the species spectrum of bark and ambrosia beetles was primarily influenced by the lure ( $df=381$ ,  $F=20.30$ ,  $p=0.002$ ) and secondarily by the day of the season ( $df=381$ ,  $F=6.10$ ,  $p=0.025$ ; Figures 6A,B).

## 4. Discussion

Sulcatol has been reported as a potential aggregation pheromone of *G. materiarius* (Flechtmann and Berisford, 2003). This is likely because sulcatol has been detected in the guts of males that initiate attacks on the host tree and ceases production after mating with females (Flechtmann and Berisford, 2003). However, we captured more *G. materiarius* in ethanol-baited traps than in WSL-baited traps (Table 2). The details and chiral ratio of sulcatol in WSL are unknown to us, while the chiral ratio of the pheromone observed from the headspace of *G. materiarius* was 31% (S)-(+)- and 69% (R)-(–)-sulcatol (Flechtmann and Berisford, 2003). No *G. materiarius* has been caught on sulcatol in the USA (Miller and Crowe, 2020).

Ethanol alone significantly attracted all ambrosia beetles. It is well-established that ethanol is the primary volatile lure for ambrosia beetles (Kühnholz et al., 2001; Kelsey and Joseph, 2003; Ranger et al., 2013, 2019, 2021; Supplementary Table S2). Therefore, it was expected that ethanol would have a higher capture ratio of invasive ambrosia beetles compared to the other lures.

Among the 46 bark and ambrosia beetle species detected, *C. bodoanum*, *G. materiarius*, *X. attenuatus*, and *X. germanus* are the invasive ambrosia beetle species that were expected to be found. These



species were identified in a total of 187 individuals, which accounts for less than 3 % of the total number of bark and ambrosia beetles collected. This suggests that the population densities of these beetles are still relatively low. In comparison, Slovakia and Slovenia have reported higher captures of the invasive ambrosia beetle *X. germanus* (Galko et al., 2018; Franjević et al., 2019), suggesting that the Czech Republic is on the edge of the range of the species' range. Similar observations apply to *C. bodoanum* (Fiala et al., 2021) and *G. materiarius* (Fiala, observ.). *X. attenuatus*, on the other hand, is widespread throughout the Czech Republic but is not abundant (Fiala and Holuša, 2024), which explains its relatively low number of individuals in the current study.

Europe is home to approximately 30 species of invasive Scolytinae, with most of them belonging to the ambrosia beetle group (Marchioro et al., 2022; Alonso-Zarazaga et al., 2023). These ambrosia beetles are predominantly native to temperate or subtropical forests, making them particularly concerning for southern Europe regions due to similar climatic conditions (Francardi et al., 2017; Leza et al., 2020). In the Central and Northern European countries, damage has been recorded only for *X. germanus*, while other species have not caused significant harm (Maksymov, 1987; Galko et al., 2019). *X. attenuatus* is considered a secondary pest (Borowski et al., 2012; Skrylnik et al., 2019). As for *C. bodoanum*, no damage has been documented in Europe; and *G. materiarius* is also not known to cause damage in the region (Mazur et al., 2018). Nevertheless, it is important to note that all these ambrosia beetles can serve as vectors for fungi that can be detrimental to trees, so their occurrence should not be ignored (Batra, 1963; Nakashima et al., 1992; Kawasaki et al., 2010; McPherson et al., 2013; Moore et al., 2019).

The occurrence of all species of invasive ambrosia beetles, as well as most of the other bark and ambrosia beetles, was observed at a single locality (Kdyně). This can be probably attributed to the presence

of diverse range of tree species, both deciduous and coniferous, in the surrounding area [e.g., *Quercus robur* L., 1753, *Larix decidua* Mill., 1768, *Fagus sylvatica* L., 1753, *Abies alba* Mill., 1768, *Populus tremulae* L., 1753, *Sorbus aucuparia* L., 1753, *Acer pseudoplatanus* L., 1753, *Pinus sylvestris* L., 1753, *Corylus avellana* L., 1753, *Picea abies* (L.) H. Karst, 1881].

In all conducted analyses, the day of season emerged as a significant factor, with the highest number of captured beetles observed in May during the study (as shown in Figures 2, 3). This pattern can be attributed to the increased flight activity of the most abundant beetle species during the period (Pfeffer, 1989). Notably, traps baited with Cembrawit exhibited a significant peak in summer, which can be attributed to the captures of *Ips cembrae*, with most often two generations per year in Central Europe (Holuša et al., 2014). The observed variation in species richness among the different localities can be attributed to the variations in tree species composition across the study area.

A total of 10 species of bark and ambrosia beetles were captured across all locations (*Anisandrus dispar*, *Crypturgus cinereus*, *Dryocoetes autographus*, *Hylastes attenuatus*, *Hylastes cucicularius*, *Ips typographus*, *Pityogenes chalcographus*, *Pityophthorus pityographus*, *Taphrorychus bicolor*, and *Xyleborinus saxesenii*). The species are commonly found in Central Europe (Pfeffer, 1989). Notably, two of these species, *I. typographus* and *P. chalcographus*, have recently experienced severe outbreaks and have become epidemic in the studied area (Fiala and Holuša, 2022). The occurrence of most of the species was observed in two or three localities, depending on the presence of their respective host trees. For instance, *Pityokteines spinidens* and *Pityokteines vorontzowi* were predominantly found in sites with *Abies alba* Mill. Another group of species was present in several localities but was captured in only a few specimens. An example is *Phloeotribus spinulosus*, which primarily develops on thin



dead spruce twigs and is exclusively found on them (Pfeffer, 1989). In our experiment, this bark beetle was detected in three localities, with a total of 5 specimens caught. It can be speculated whether this occurrence is influenced by the *I. typographus* outbreak and the availability of abundant suitable breeding material, as observed in other species (Fiala and Holuša, 2021, 2022), or if it represents a long-term trend.

Relatively low catches of certain bark beetle species can be attributed to specific factors. *Cryphalus piceae* is generally a rare inhabitant of fir forests (Procházka and Schlaghamerský, 2019), and the occurrence of fir trees is limited in the Czech Republic (Kozáková et al., 2011). *Dryocoetes hectographus* primarily occurs in mountain spruce forests (Pfeffer, 1989), where traps were not placed. Only two specimens of the bark beetle *Dendroctonus micans* were caught in the Kladská locality because this species is not attracted to traps (Procházka et al., 2014). Although substances such as E-conophthorin, exo-brevicommin, and ipsdienol elicit a positive response from *D. micans*, they are most likely not its pheromones (Tømmerås et al., 1984; Zhang et al., 2002). Contrarily, the substance exo-brevicommin has shown effectiveness in trapping American and Asian species of the *Dendroctonus* genus (Barclay et al., 1998; Greenwood and Borden, 2000; Zhao et al., 2017). However, the fertilized females of *Dendroctonus* emerge from galleries and primarily rely on visual detection during host tree attacks, suggesting that pheromones may not play a significant role for them, except during the larval stage (Lukášová and Holuša, 2011).

An association between  $\alpha$ -pinene and WSL had a significant effect on attracting conifer species of bark beetles that typically inhabit areas where pine bark meets the soil, such as *Hylastes angustatus* and *Hylastes attenuatus* (Schroeder and Lindelöw, 1989; Erasmus and Chown, 1994). While aggregation pheromones *cis*-verbenol and *trans*-verbenol have been reported for *T. piniperda* (Kangas et al., 1970), it has been suggested that *T. piniperda* relies on monoterpenes, including kairomones derived from its host, pine, for host and mate location (Lanne et al., 1987). This explains the high attractiveness of  $\alpha$ -pinene observed in our experiments, as all the species live on pine trees (Byers et al., 1985; Schroeder and Lindelöw, 1989; Czokajlo and Teale, 1999; Poland et al., 2004).

In Cembräwit, the main component is ipsdienol, which serves as an aggregation pheromone for *Ips typographus* and *Ips cembrae* in low concentrations (Vité et al., 1972; Stoakley et al., 1978). Therefore, it is not surprising that most specimens of both bark beetles were caught in traps baited with Cembräwit (see also Grucmanová et al., 2014; Holuša et al., 2014). *Pityokteines vorontzowi* was also significantly attracted to Cembräwit, as it shares ipsdienol and ipsenol as its aggregation pheromones (Harring, 1978). The highest number of bark beetles caught was observed on Cembräwit® compared to other lures, which can be attributed to the aforementioned outbreak of *I. typographus* in the study area (Fiala et al., 2022).

Ethanol and WSL significantly attracted two species of ambrosia beetles, *Xyleborinus saxesenii* and *X. germanus* as well as two species of bark beetles, *Dryocoetes autographus* and *Hylurgops palliatus*. These species have been frequently observed in traps baited with ethanol (Schroeder and Lindelöw, 1989; Lindelöw et al., 1993). More species of bark and ambrosia beetles responded to these lures compared to any other lure.

The ambrosia beetle *Trypodendron lineatum* was significantly associated with three lures,  $\alpha$ -pinene, ethanol, and WSL. This is

consistent with previous studies that have shown  $\alpha$ -pinene and ethanol to be attractants for this species (McLean and Borden, 1977; Schroeder and Lindelöw, 1989). Conversely, this ambrosia beetle exhibits weak attraction to the substances used in Cembräwit, such as ipsdienol and methylbutenol (Gavvalis et al., 1981). The association of Cembräwit, ethanol, and WSL significantly attracted only the bark beetle *Orthotomicus laricis*. Bark beetles of the entire genus *Orthotomicus* are primarily attracted to ipsdienol, *cis*-verbenol, and 2-methyl-3-buten-2-ol (Giesen et al., 1984; Valkama et al., 1997). Ipsdienol is the main component of Cembräwit®, which also contains ipsenol, methylbutenol, and amitinol (Zuhlke and Mueller, 2008). However, since *O. laricis* is found in the tops of uprooted spruces (Fiala, observ.), on pine stumps, and on felled spruce wood that is shaded (Holuša et al., 2017, 2019), it is evident that this bark beetle is also attracted to ethanol.

Overall, the results demonstrate that accounting for the influence of location and day of the season, the differences among lures were significant (see also Beaver and Löytyniemi, 1991). Ethanol significantly attracted all ambrosia beetles in our experiments. In the USA and Italy, volatile lures such as ethanol, a combination of  $\alpha$ -pinene and ethanol, and other combinations of ipsdienol+*cis*-verbenol+methylbutenol are used for monitoring invasive bark beetles (Rassati et al., 2015; Rabaglia et al., 2019). In Italy, these traps are placed in ports and adjacent forests; with higher capture success observed in deciduous forests compared to coniferous forests or ports (Rassati et al., 2015). Our experiment also shows the higher capture success of invasive ambrosia beetles was in deciduous forests, as evidenced by the capture of 162 specimens (e.g., 87%) at the Kdyně locality, probably due to the prevalence of deciduous trees in that area. A similar approach is employed in New Zealand, where traps are placed in ports, international airports, and adjacent forests using different lure combinations such as  $\alpha$ -pinene+ethanol,  $\beta$ -pinene+ethanol, frontalin+ethanol, and ipsdienol. This monitoring model has proven successful in the early detection of invasive bark beetles, increasing the chances of eradication when infestations are still relatively small (Brockerhoff et al., 2006b). In Australia, the monitoring efforts have a broader focus and include the capture of Lepidoptera. Various lures such as ethanol, cineole,  $\alpha$ -pinene, phellandrene, and a combination of pinene, phellandrene, cineole, terpene, and cymene as bait are used. Traps are positioned near ports and airports and within a 5 km radius of these areas (Bashford, 2012). In France, monitoring activities targeting invasive cerambycids include testing trapping methods using  $\alpha$ -pinene+ethanol in Ecotrap traps. Traps were deployed in natural forests and later extended to ports, airports, and horticulture areas (Fan et al., 2019). In all these cases, the traps successfully captured invasive species.

There are several methods available for monitoring bark and ambrosia beetles, with baited traps being the most effective among them. Baited traps offer several advantages, including their ability to cover large areas, easy application in various locations, and cost-effectiveness. However, they also have some drawbacks, primarily related to the selection of bait types and the physical demands associated with checking numerous traps and subsequent determination in the laboratory (Poland and Rassati, 2019). While citizen science can help alleviate the physical demands of monitoring (Steininger et al., 2015), coordination is still necessary, and the expertise of bark beetle specialists will always be essential for accurate determination.

## 5. Conclusion

Ethanol serves as a universal lure that effectively attracts both bark and ambrosia beetles, with the majority of Scolytinae species being caught in traps baited with ethanol. Conversely, ethanol's inability to attract economically important species in Central Europe, such as *Ips typographus*, is a significant advantage since these species are typically abundant. By not attracting them, the number of bark and ambrosia beetles captured is not needlessly increased, thus reducing the complexity and cost associated with sorting and determination. For the detection of invasive ambrosia beetles in Central Europe, it is recommended to use one bait of ethanol per year installed in April in any impact trap. Our preference lies with Ecotrap® due to its disassemblability, storage capacity, and ability to catch beetles in a dry state without the need for preservative liquid. Moreover, the material remains intact by predators even after a two-week period.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

JH: Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. TF: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. PP: Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

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## Conflict of interest

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

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2023.1258729/full#supplementary-material>

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